Understanding the Nutritive Value of Pasture Mixtures

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Abstract of a Dissertation submitted in partial fulfilment of the requirement for the Degree of Bachelor of Agricultural Science

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By

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An investigation of how nutritive value changes with respect to the number of species and proportions of species in pasture mixtures was carried out at Lincoln University. Tetraploid perennial ryegrass cv. Base, plantain cv. Tonic, white clover cv. Apex and red clover cv. Grasslands Sensation were all grown in monocultures, two species, three species and four species mixtures based on a simplex centroid mixture experiment design. The aim of the experiment was to quantify the nutritive value of each of these mixtures to be able to define an optimum pasture mixture based on the four species used. Therefore, the species contribution to nutritive value and diversity effects were identified. Models have been produced to predict nutritive value attributes of a mixture involving the four species investigated.

The optimal mixture was defined based on parameters of maximising annual dry matter yield and annual yields of crude protein and metabolisable energy while meeting target annual mean concentrations of metabolisable energy, acid detergent fibre, neutral detergent fibre and crude protein based on animal requirements. This mixture comprised of the following proportions: 0.43 ryegrass, 0.20 white clover and 0.37 red clover based on the number of seeds/m^2. Which was equivalent to the sowing rate of 12.90 kg/ha of ryegrass, 1.50 kg/ha of white clover and 6.48 kg/ha of red clover and a total sowing rate of 20.88 kg/ha. This mixture would allow for the pasture to meet animal requirements while not limiting intake or stocking rate. The optimal pasture mix was expected to have an annual yield of 14,250 kg DM/ha, annual metabolisable energy yield of 160 GJ/ha and an annual crude protein yield of 2806 kg/ha. It was also expected to have metabolisable energy concentration of 11.2 MJ ME/kg DM, an acid detergent fibre concentration of 264 g/kg DM, a neutral detergent fibre concentration of 380 g/kg DM and a crude protein concentration of 195 g/kg DM.

Nutritive value was found to be dependent on the species present and their proportions in each mixture. Nutritive value has shown variation across years and seasons which showed that diversity effects among species were not consistent across time. The dynamic systems of pasture mixtures show that proportions of species do not remain consistent across time. Therefore, the diversity effects were not consistent across time either. Proportions of species did not remain consistent with the sown proportions with white clover being most outcompeted within the sward often stabilised at 10% of the mixture. The proportions of ryegrass, plantain and red clover varied depending on the mixture. The nutritive value was subsequently remodelled based on the actual proportions of species in the mixtures. In conclusion, the nutritive value of the pasture mixtures were dependent on the species present and the proportions of each species. The proportions of species changed across seasons due to growth patterns and overs years as a result of the succession of species.

Keywords: dry matter yield, *Lolium perenne*, nutritive value, pasture mixtures, perennial ryegrass, *Plantago lanceolata*, plantain, red clover, simplex design, *Trifolium pratense*, *Trifolium repens*, white clover.

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1 INTRODUCTION

Pasture renewal requires a pasture mixture to be formulated based on the needs of the farmer and to match the farm system. Pasture mixtures, compared with monocultures, allow for species to access different resources with respect to nutrients and light (Harris, 2001), reducing competition with the ability to produce synergistic relationships. Diversity effects, the increase in yield above the mid-species mean, can be seen when mixing perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) compared to the respective yields of each monoculture (Harris, 1968). Stewart *et al*. (2014) proposed 10 essential questions for selecting a pasture mix, focussing on the environmental conditions, stock type and timing of sowing and when the feed is needed. Therefore, evaluation of pasture mixtures is necessary to identify the optimum mixture relative to the environment and stock type which will determine the pasture species to be used in the mixture.

Typical pasture mixtures in New Zealand agriculture include perennial ryegrass and white clover, while plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) have started to become included in mixtures more frequently. Traditionally, pasture mixture formulation has focused on maximising yield and dry matter, which is often paired with a reduction in nutritive value. As the pasture mass increases, the concentration of crude protein declined while acid detergent fibre (ADF) and neutral detergent fibre (NDF) increased (Lee *et al*., 2015). Hence, the optimal mixture for yield, may not be the same as the optimal mixture for nutritive value due to the trade-off in characteristics. Both yield and nutritive value may be affected by species identity effects and the species interaction effects. The net outcome of the species interaction effects is the diversity effect. Species identity and interaction effects are scaled by the relative abundance of species on the mixture. Mixing species can be synergistic or antagonistic depending on the species interactions. The diversity effect is the result of all of these factors. Mixture creation have previously been carried out to quantify yield in multispecies grasslands (Connolly *et al*., 2009; Kirwan *et al*. 2007; Nyfeler *et al*. 2009) and to formulate optimal seed mixtures (Harris, 1968; Harris, 2001; Ryan-Salter & Black, 2012). Creating a mixture which can maintain nutritive value, whilst being high yielding would allow for animal carrying capacity and production to be maintained or improved.

Nutritive value is important for animal growth and production. Improving animal production can be crucial during the summer period where the nutritive value of a perennial ryegrass-white clover sward is known to be limiting. Previous studies by Hutton *et al*. (2011), Somasiri *et al*. (2016), Kenyon *et al*. (2017) and Golding *et al*. (2011) have shown that pasture mixtures, particularly those which are herb clover mixtures, can support higher animal production than standard perennial ryegrass-white clover pasture. Lambs tended to show significant increases in live weight gain ranging from 11% to 52% while grazing the herb clover pasture compared to the perennial ryegrass-white clover mixtures during the summer period. However, dairy cow milk production does not show the same trend with little increase in milk production from grazing pastures with a higher legume and herb proportion (Edwards *et al*., 2015; Soder *et al*., 2006; Woodward *et al*., 2013).

Monocultures of individual species are often used to evaluate nutritive value of pasture. Combining species into a mixture, followed by nutritive value analysis may help to understand species interactions. Nutritive value studies can be limited in the number of harvests which contribute to the result of the study. This assumes that nutritive value of a pasture remains consistent across time. Sturludóttir *et al*. (2013) evaluated nutritive value of grassland communities at five different European locations and one Canadian location where harvests were taken twice per year for 3 years allowing for some of the seasonal and annual differences to be accounted for. Delaby *et al*. (2010) looked at nutritive value across an entire year at two different European locations. The harvest regime was based on the cutting typical management of the Northern Hemisphere pastures. This does not directly correlate with the higher frequency of harvesting in a grazing situation seen in the southern hemisphere. However, the measures of nutritive value can be used to predict the nutritive value of pastures under different harvest regimes.

Pasture establishment methods can influence the interaction between pasture species. Traditional establishment methods sow all species together in the drill rows. Other options such as separating species in space through alternate drill rows (Hurst *et al*., 2000), diamond drilling (Thom & Ritchie, 1993) or cross drilling (Thom & Bryant, 1996) can alter the interaction and competition between species. Spatial separation of species in alternate drill rows tends to benefit slow establishing species, such as white clover when sown with perennial ryegrass, reducing the competition for light and resources. However, the long-term interaction (beyond the first production year) between these separations and the influence on nutritive value has yet to be evaluated.

Mixture experiments can be designed as simplex lattice or simplex tetrahedron designs depending on the number of components and desired mixtures (Cornell, 2002). These mixture designs eliminate the potential for confounding as the mixtures can be evaluated with respect to their respective components. Pasture mixture experiments that do not incorporate all the monocultures of the species used have the potential to have confounded results (Nobilly *et al*., 2013; Woodward *et al*., 2013). The diverse pasture may not have better production than a monoculture of a species involved but has better production than a standard perennial ryegrass-white clover pasture used for comparison.

The aim of this experiment was to understand the nutritive value of pasture mixtures across seasons and years. The subsequent objective of this research was to quantify the nutritive value of pasture mixtures and identify an optimal seed mixture that would maximise nutritive value while not restricting pasture yield. The optimal seed mixture can then be defined using annual pasture yield, metabolisable energy yield, crude protein yield, metabolisable energy concentration, acid detergent fibre concentration, neutral detergent fibre concentration and crude protein concentration. This mixture should be high yielding with a high nutritive value. The models formed, based on the interactions among species with respect to the monocultures, could subsequently be used to predict the nutritive value of pasture and/or seed mixtures grown in a Canterbury dryland environment. Which allows for commercial mixtures to be evaluated with regards to their potential nutritive value based on the sown proportions of species.

The hypotheses of this experiment were that the proportions of species would determine nutritive value, proportions of species would remain consistent across time, keeping nutritive value constant and spatially separated pastures are be more stable across time. This dissertation includes a review of the literature surrounding pasture nutritive value of individual species and mixtures, sowing methods, near infrared spectroscopy analysis techniques and mixture experiments; materials and methods of the conducted study; results and a discussion to understand the nutritive value of pasture mixtures across seasons and years.

2 REVIEW OF THE LITERATURE

2.1 Introduction

Pasture mixtures and pasture establishment methods can influence the pasture production and relationships between the sown species. Mixture formulation tends to be focused on maximising yield which often has a negative impact on the nutritive value. There is likely to be a trade-off between these two functions which results in the overall animal production that can be observed. The aim of this review was to evaluate the basis of pasture mixture formulation, how animal production differs as a result of grazing diverse pastures compared to a perennial ryegrass-white clover pasture, the nutritive value of individual species and mixtures, sowing methods for pasture establishment, quality analysis techniques and designs of mixture experiments.

2.2 Pasture mixtures

The total number of species available to farmers can influence decision making relative to the number of species sown when establishing a new pasture. Harris (2001) reported that in 1998 farmers had the choice of 18 perennial ryegrass cultivars and 73 grass species cultivars in total. While there were 13 white clover cultivars, four herb cultivars and 40 legume and herb cultivars in total. Levy (1970) recommended sowing eight different pasture species in a new pasture following a primary scrub burn. Similarly, the permanent long rotation pasture recommended by Levy (1970) contained eight different cultivars (two long rotation ryegrasses, perennial ryegrass, timothy (*Phleum pratense*), cocksfoot (*Dactylis glomerata*), crested dogstail (*Cynosurus cristatus*), red clover and white clover) with a total sowing rate of 40 lb/acre (45 kg/ha). The number of species present in the mixture was aimed to maximise the early growth of the pasture with the potential for succession by the slower establishing species. The long rotation ryegrass would provide early growth before the slower establishing species (cocksfoot, timothy and white clover) began producing sufficient yield providing they were not outcompeted in the early stages of establishment and made up a sufficient proportion of the sward. Levy (1970) did not revisit his pasture mixture recommendations following establishment to assess the botanical composition of the sward and the contribution each of these sown species was providing to the yield of the pasture.

Past and present pasture mixture formulation tends to comprise perennial ryegrass and white clover. Leading seed companies in New Zealand recommend one or two cultivars of perennial ryegrass, either two diploids or a diploid and a tetraploid, with two cultivars of white clover for a dairy pasture (Table A1). In comparison, sheep pastures typically have one cultivar of perennial ryegrass, either diploid or tetraploid, with one or two cultivars of white clover and either red clover, chicory (*Cichorium intybus*), plantain or cocksfoot (Table A2). These pasture mixtures have a reduction in the number of species used compared to recommendations by Levy (1970). The introduction of domesticated herb species, which are becoming more popular in pasture mixtures, has extended the range of species which have the potential to be included in mixtures. Mixtures have the potential to include up to six species but have little evidence behind the inclusion of more species based on the production environment. Most pasture mixtures tend to be formulated for the purpose of maximising yield. There is a trade-off between yield and nutritive value. As yield increases nutritive value decreases (Lee *et al*., 2015; White & Hodgson, 1999). Due to the increase in ADF and NDF in the plant, there is a reduction in the organic matter digestibility (OMD) and metabolisable energy (ME). Measuring yield to determine animal production is a process which many farmers already carry out but the increased yield of a pasture is not likely to directly correlate to increased animal production. Formulation of pasture mixtures on the basis of nutritive value, with respect to proportions of species sown, is not highly researched. However, some mixtures are said to be of higher nutritive value than others but contain the same species, just different cultivars. Therefore, further research could compare the interactions between species as a function of nutritive value, with the potential to determine the optimal pasture mix to maximise animal production through nutritive value.

Diversity effects are a sum of the interactions between species to provide a benefit to the output of the pasture. This is typically seen through the combination of ryegrass and white clover to give an increase in yield above the mid-parental mean. Harris (1968) showed the diversity effect through this mixture (Figure 2.1) increasing the proportion of clover can create a diversity effect and improve the overall yield of the mixture. The difference in resource use of the two species was attributed to the majority contributing factor to the diversity effect. Comparatively, species with relatively similar attributes were also tested. 'Manawa' and 'Ruanui' ryegrasses were combined and there was a reduction in yield compared to the respective monocultures. Therefore, species with similar characteristics and resource use are not likely to show a diversity effect. Diversity effects within nutritive value studies have not been investigated. These would test whether species with little similarity in nutritive value would display the same diversity effect as shown by Harris (1968) with yield.

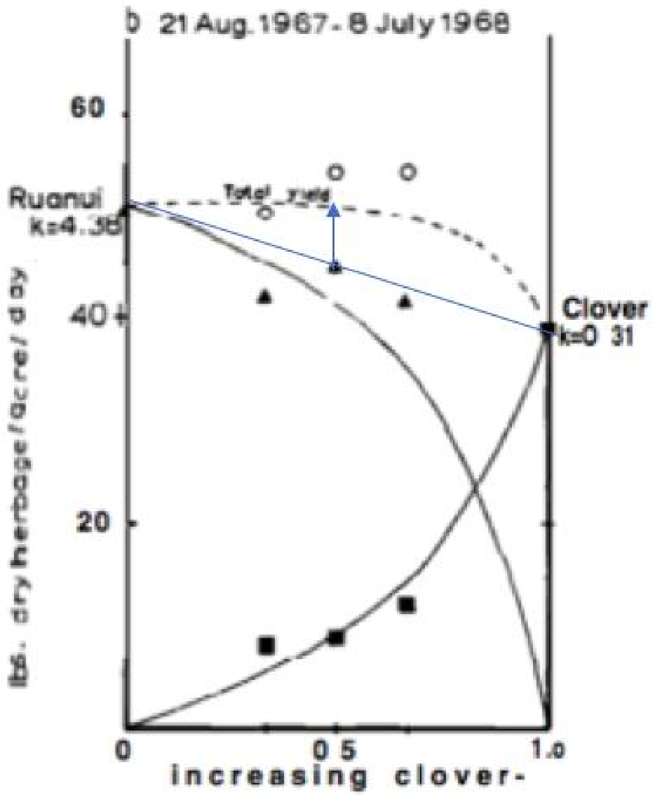

Figure 2.1: Replacement diagram based on the yield of 'Ruanui' ryegrass and white clover in the second year. (Curve fitted assuming a linear increase of the monoculture yield in relation to an assumed linear increase of nitrogen supply with increasing clover). The straight line represents the mid-species mean and the arrow represents the diversity effect. (Adapted from Harris, 1968).

Abundance refers to the total amount of each species present within a mixture. Studies such as Ryan-Salter & Black (2012) and Sturludóttir *et al*. (2013) tested the effect of sowing species at two levels of abundance. Sowing species at a higher level of abundance (e.g. twice the recommended sowing rate) did not increase yield in either study. The increased abundance of species led to increases competition for the same recourses with the same mechanism to acquire these resources. Therefore, increased abundance of mixtures did not result in increased yield or a diversity effect.

Current commercial sowing rates of pasture mixtures can range from 20-40 kg/ha. The ryegrass component of the sward typically comprises 16-30 kg/ha while white clover is usually 4-5 kg/ha. Therefore, ryegrass can be sown at a rate four times higher than white clover. By describing the proportions of species in a pasture mixture as the seeds/m^2 provides a more accurate representation of the ratio of one species to another rather than comparing in terms of sowing rate (kg/ha). Based on seed count per m^2 using average thousand seed weights (TSW) from White & Hodgson (1999) were 2.0, 0.9, 0.6, 1.9, 1.2 and 2.0 g for diploid perennial ryegrass, cocksfoot, white clover, red clover, chicory and plantain, respectively. Seed counts based on 20 kg/ha diploid ryegrass, 3 kg/ha cocksfoot, 4 kg/ha white clover, 4 kg/ha red clover, 2 kg/ha chicory and 1 kg/ha of plantain can be calculated as 1000, 333, 667, 211, 167, 50 seeds/m^2 for each species respectively. At these sowing rates ryegrass makes up 41.2% of the total seed/m^2, cocksfoot 13.7%, white clover 27.5%, red clover 8.7%, chicory 6.9% and plantain 2.1%. In this pasture mixture, there is dominance by the grass species (54.9%) over the legumes (36.2%). Therefore, sowing the ryegrass at a lower rate such as 15 kg/ha reduces the dominance of the grass (49.8%) over the legumes (40.3%). Removing the cocksfoot has a similar effect (47.8% compared to 41.9%) on the dominant component of the sward. Evaluating mixtures in terms of seeds/m^2 allows for better evaluation of the dominant species in the pasture mixture. Therefore, seeds/m^2 can then be used to understand whether the mixture is suitable for the desired purpose.

Species complementarity in a pasture mixture allows for the majority of species to be ready for grazing at the same time. Species structure determines whether they are better suited to set-stocking or rotational grazing. Species such as red clover and chicory have a crown structure which requires rotational grazing and can become damaged if grazed too firmly, while perennial ryegrass, cocksfoot and white clover can be grazed in either set stocking or rotational situations as they are tolerant to treading. Therefore, pasture species in a mixture should be selected relative to the purpose of the pasture and minimise any differences there are in the timing of grazing. Perennial ryegrass usually forms a compatible mix with white clover and in some instances, can be combined with several other species (Stewart *et al*., 2014), due to its tolerance of different grazing systems. However, the level of suitability of the other species to the type of grazing system used may help to cause the dominance of ryegrass within the sward.

2.3 Animal production

Generating diverse pastures, which increases the number of species in the pasture, can alter animal production. Table 2.1 shows a summary of the production change of animals grazing diverse pasture compared to simple pastures. Animal production can vary depending on the pasture species being grazed and animal intake. Milk production changes ranged from -6% to +10%. The differences in production may be a result of the presented measure of milk and the length of time of the study. Comparatively, Woodward *et al*. (2013) also measured production across three seasons (measurements taken as totals for spring, summer and autumn in the first two seasons and spring and summer in the final season) and found no difference in milk solid production. They attributed the lack of difference to be associated with there being no difference in ME between the simple and diverse pastures. Other differences in milk production presented in Table 2.1 were a reduction in milk solid production. No differences in intake were observed between the simple and diverse pastures which were classed as the reason for a negligible change in production.

Herb clover pastures are also known for their high nutritive value and crude protein content compared to perennial ryegrass-white clover pastures. These mixtures typically contain herbs such as plantain and chicory with legumes such as white clover and red clover. Hutton *et al*. (2011) demonstrated 17-25% higher ewe milk production and 10.3% higher lamb survival of sheep grazing herb-clover pastures (Table 2.1). These pastures comprised of chicory, plantain, white clover and red clover. The increased survival was attributed to the lambs drinking more milk as a result of increased milk production. However, the cause of the increase in milk production was not identified. Studies by Kenyon *et al*. (2017), Somasiri *et al*. (2015b) and Golding *et al*. (2011) all looked at the growth rate of weaned lambs while grazing herb-clover pastures. Kenyon *et al*. (2017) investigated a plantain, white clover and red clover pasture alongside a chicory, plantain, white clover and red clover pasture. These were compared with perennial-ryegrass white clover pastures. The chicory, plantain, white clover and red clover pasture produced 70 g/d higher live weight gain compared to the perennial ryegrass-white clover pasture. Somasiri *et al*. (2015b) investigated the influence of three different pasture mixtures on the live weight gain of weaned lambs. The standard reference pasture contained perennial ryegrass and white clover, the plantain mix contained plantain, white clover and red clover while the chicory mix contained chicory, plantain, white clover and red clover. Lamb live weight gain was highest when grazing the chicory pasture at 44.5 g/d higher than the pasture mixture. However, the plantain mixture did not show significant differences in lamb growth rate compared to a chicory pasture. Therefore, Somasiri *et al*. (2015b) concluded that either the plantain or chicory mix would be superior to the standard perennial ryegrass-white clover pastures to boost lamb live weight gain. In order to get test the benefit of these mixtures, it would be worthwhile to evaluate these pasture mixtures in temperate regions elsewhere in New Zealand and the world. Lastly, Golding *et al*. (2011) compared an old pasture, a new pasture (tetraploid perennial ryegrass and white clover), a plantain pasture (tetraploid perennial ryegrass, plantain and white clover) and a herb clover pasture (plantain, white clover, red clover). Animal live weight gains did not differ between the new and old pastures. However, the herb clover pasture had a 128 g/d higher live weight gain than that of the pasture mixtures while the plantain pasture supported less live weight gain than the new and old pastures. The lack of difference among the new, old and plantain pasture was attributed by Golding *et al*. (2011) to the plantain being selectively grazed against in the autumn period as it became unpalatable. Therefore, the higher production from the herb clover pasture is likely to be a response to the clovers being grazed in the autumn and their higher nutritive value.

Calculation of animal requirements for growth and maintenance has typically been based on their protein and ME requirements. Growth requires higher intake and nutrients than maintenance. Nicol & Brookes (2007) and Brookes & Nicol (2007) provided calculations of the ME and CP requirements of livestock. These calculations are based on the sex, live weight, growth rate, pregnancy and lactation demands. To enhance decisions on allocation of feed accurate nutritive value of pasture is essential to ensure the animals are meeting their requirements. Although ME and CP may be the two most important functions of animal growth and production, additional components such as ADF and NDF requirements of the animal could be beneficial to formulate the optimal pasture for animal growth and production.

Table 2.1: Summary of the production change between simple and diverse pastures for different classes of stock.

Stock class	Production change	Reference	Potential cause
Dairy cow	+1.7 L/d (+10%) * -0.08 kg MS/d (-5%)	Totty *et al*., 2013	No difference in intake
Dairy cow	No difference in production averaged across three years	Woodward *et al*., 2013	No difference in energy from swards being grazed
Dairy cow	+0.5 kg milk/d (+1%)	Soder *et al*., 2006	No difference in dry matter intake
Dairy cow	-1.3 kg MS/cow/d (-6%)	Edwards *et al*., 2015	Negligible differences in dry matter intake and nutritive value
Ewe	17-25% higher milk production *	Hutton *et al*., 2011	
Lambs	+10.3% greater lamb survival	Hutton *et al*., 2011	Lambs consuming more milk
Weaned lambs	+70 g/d (+24%) *	Kenyon *et al*., 2017	ME differences
Weaned lambs	+44.5 g/d (+21%)	Somasiri *et al*., 2015b	Pasture quality
Weaned lambs	+128 g/d (+52%) *	Golding *et al*., 2011	Plantain being selectively grazed against during autumn
Yearling lambs	+38.2 g/d (+ 11%) *	Somasiri *et al*., 2016	Higher quality of herbage consumed

* Studies which showed significantly higher production when grazing a diverse pasture compared to a simple pasture.

2.4 Nutritive value

Components of nutritive value measurements include: total N, dry matter (DM)/moisture content, CP, NDF, ADF, OMD, *in vitro* dry matter digestibility (IVDMD) and ME. The summation and interaction between these components dictate the overall nutritive value of the plant and the interaction between plants gives the nutritive value of the sward.

Legumes, such as white clover and red clover, are known for having greater N content than non-legumes such as grasses and herbs. Figure 2.2 shows a summary of the CP content for perennial ryegrass, plantain, white clover and red clover. Both clovers show higher CP than perennial ryegrass (mean CP content 185 g/kg DM), which is expected due to their N fixation capability. However, red clover shows a greater spread in CP, ranging from 270 to 180 g/kg DM. Vasiljević *et al*. (2011) observed the lowest value for CP (180 g/kg DM), which may be an outlier. Resulting in the lower mean CP observed for red clover. The CP value was an average of four red clover cultivars across two seasons where CP ranged from 158 to 195 g/kg DM depending on the season and the time since the previous pasture cut. Comparatively, white clover displays a consistently high CP measurement with a small range (Figure 2.2), indicating that the CP content may remain consistent throughout the year.

Plantain CP content showed a definitive split in values (Figure 2.2). The bottom proportion ranges from 141 to 173 g/kg DM while the upper proportion was from 247 to 283 g/kg DM. These higher CP values seem very high for a non-legume species, and may be a function of available N to the plant and timing of measurement as the CP concentration of a non-legume is not expected to be as high as that of a legume. Harrington *et al*. (2006) and Fulkerson *et al*. (2008) were the two studies which measured the highest values for CP at 283 and 276 g/kg DM, respectively. Fulkerson *et al*. (2008) managed their trial so that 80% of the N removed by the plant was returned in the form of inorganic fertiliser as well as irrigation applied. Therefore, the CP content of plantain was unlikely to be limited by N availability. Fulkerson *et al*. (2008) used the same management practice for the growth of perennial ryegrass, but the CP measurement was much lower at 243 g/kg DM. The high CP observed in plantain in this study was not expected by Fulkerson *et al*. (2008) who attributed the difference between species to potentially be a function of the reproductive status of the plant as CP decreases as the plant becomes more reproductive. Autumn was when the highest CP concentrations were observed in plantain which was likely to be associated with the mineralisation of N in the soil. The mean value for plantain CP cannot be used to assume that this is the CP concentration of plantain for the majority of the growing season as there was a large variation in measures. Therefore, further summary of CP content of non-legumes could be beneficial to establish the influence of N supply of CP and the variation observed among species. Overall, CP concentration seems to be high and similar for white and cloves irrespective of soil N, and can be similar between ryegrass and plantain but depends on soil N availability.

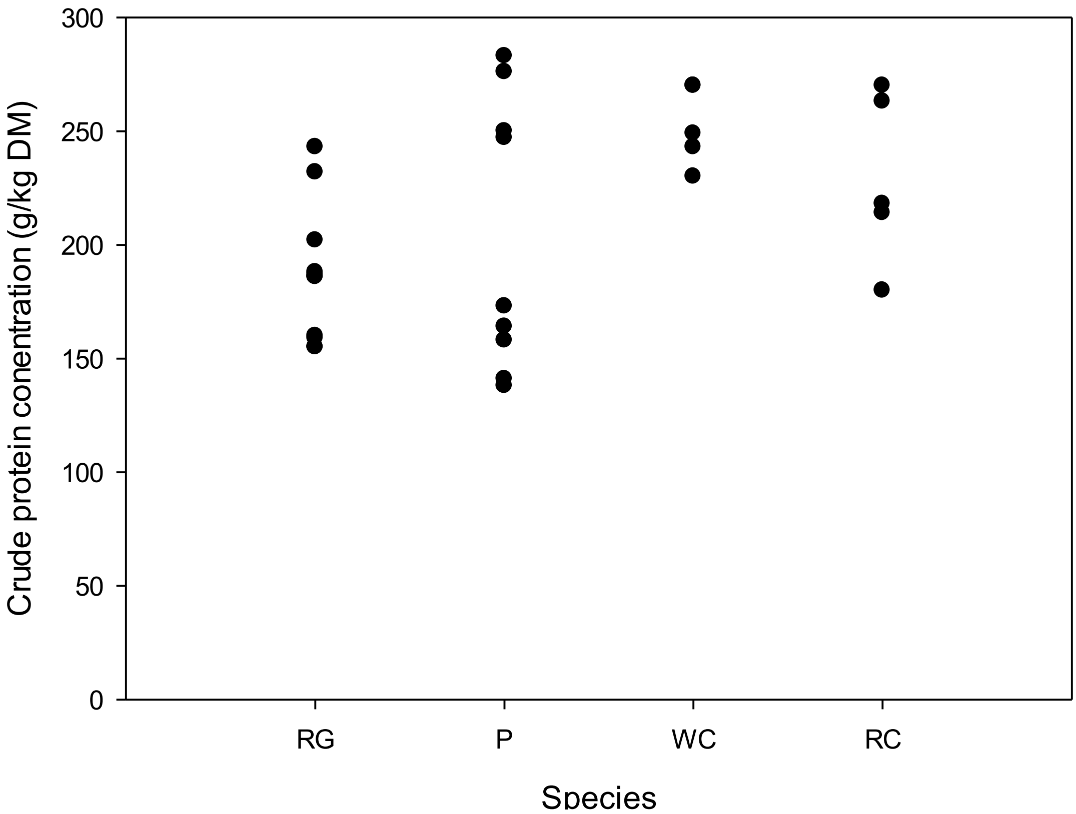

Figure 2.2: Crude protein content (g/kg DM) of perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) summarised from Tables A3, A4, A5, and A6.

The ME of a pasture refers to the energy which can be used for animal growth. Clovers tend to have a higher ME than grasses and herbs. Figure 2.3 shows a summary of ME of ryegrass, plantain, white clover and red clover. White clover had the highest ME of all the species presented while red clover has a slightly lower ME. This was likely to be associated with the structure of the red clover plant as OMD can be used to calculate ME

(McDonald *et al*., 2002). However, the ME of different species and components of these species tended to have little published literature (Litherland & Lambert, 2007; Stewart *et al*., 2014; White & Hodgson, 1999) as these values are often accepted as fact. But there may be more variation between these sources than first anticipated.

ME is related to the digestibility of the plant. Calculation of ME as a function of either OMD (McDonald *et al*., 2002) or dry matter digestibility (Fulkerson *et al*., 2008) may have consequences on the accuracy of ME. Both methods presented should reach the same ME for the pasture. However, digestibility of swards may not always be presented in the results of the study or measured in the same way among studies (*in vitro* compared to NIR). ME has been shown to be similar among species when measured as monocultures (Figure 2.3). Which shows that ME of a pasture may not be the major contributing factor to a low nutritive value pasture.

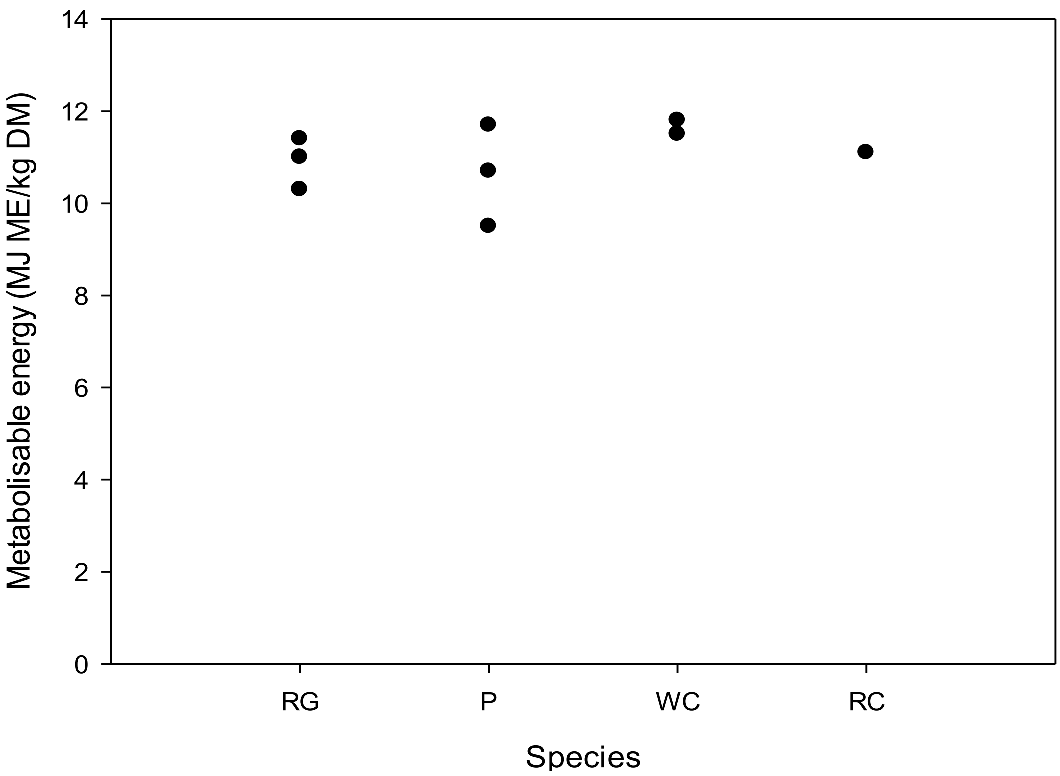

Figure 2.3: Metabolisable energy content (MJ ME/kg DM) of perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) summarised from Tables A3, A4, A5 and A6.

ADF is the least digestible proportion of the sward. It is comprised of the lignin and cellulose proportion of the plant which can give an indication of the digestibility of the plant. Both ryegrass and plantain have a range of ADF concentrations (Figure 2.4). Plant structure has a large influence on the ADF component of nutritive value. Those plants which are more erect will tend to have a greater concentration of ADF due to needing stronger structures. Hence the higher ADF of ryegrass, plantain and red clover compared to white clover. The range of values for ryegrass and plantain in Figure 2.4 likely represents the timing of harvest and measurement relative to reproductive status. The highest measure of perennial ryegrass ADF was recorded by Marley *et al*. (2005) as 363 g/kg DM. This measurement was taken in summer which is associated with when the plant is in a reproductive state. Comparatively, the lowest measure of ADF in perennial ryegrass was observed by Fulkerson *et al*. (2008) as 238 g/kg DM. This observation was an average from one measurement taken each season throughout a year. The highest ADF value was observed in summer (313 g/kg DM) and lowest in winter (232 g/kg DM).

Similar to ryegrass, plantain ADF also showed large variation among studies, with the highest measure of 340 g/kg DM observed by Derrick *et al*. (1993). This measurement was also taken in the summer, further showing the relationship between reproductive status and ADF concentration in the plant. This summer period tends to be the only highly elevated measure of ADF compared to the rest of the year. Red clover ADF measurements show less variability than ryegrass and plantain but are not as concise as white clover. Plant structure is shown to have an influential factor, as red clover has an erect stem whereas white clover has horizontal stems. This link between plant structure and ADF concentration is not as evident as the influence of reproductive status. White clover may not be influenced to the same extent as ryegrass and plantain by reproductive status as the measurements taken by Marley *et al*. (2005) were from the summer period. White clover potentially showed less variation in ADF concentration due to the plant being predominantly leaf and leaf stem whereas all other species have erect stems.

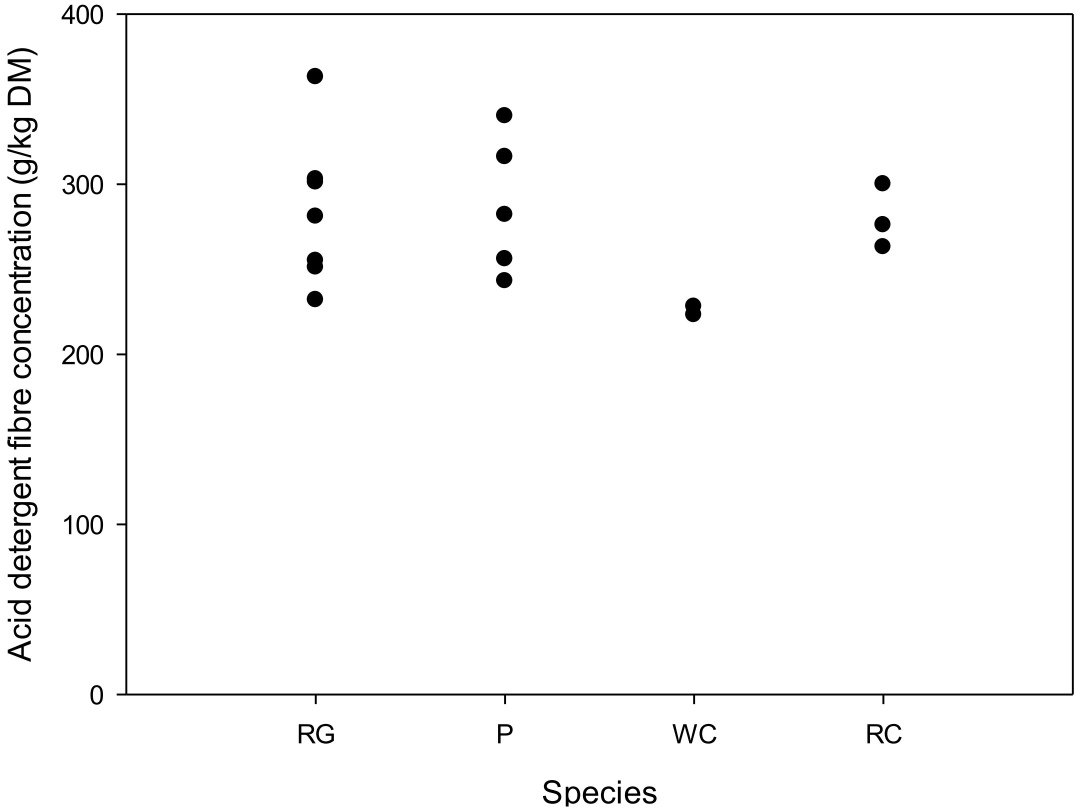

Figure 2.4: Acid detergent fibre content (g/kg DM) of perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) summarised from Tables A3, A4, A5 and A6.

NDF governs animal intake, with the higher concentration of NDF in the plant, the lower the voluntary intake by the animal. NDF is composed of ADF and hemicellulose. Increases in NDF and ADF negatively impact intake, digestibility and ME. NDF should always be greater than ADF, but the amount of hemicellulose in the plant will govern how this interaction occurs and how much higher NDF is above ADF. Figure 2.5 shows a similar relationship to Figure 2.4 where perennial ryegrass and plantain NDF is greater than the clovers. However, ryegrass NDF is much less spread out while plantain remains with a wide range of values for NDF. Unfortunately, in Tables A3 to A6 of the ADF and NDF measurements are not both presented. Therefore, it is difficult to establish whether the proportion of hemicellulose in the plant is consistent among measurements. The variability in the plantain measurements indicates that it is not. Like ADF, NDF is likely to be dependent on the reproductive status of the plant. From the presented results of plantain, Fulkerson *et al*. (2008) and Hayes *et al*. (2010) presented both ADF and NDF. The hemicellulose content was 157 g/kg DM and 130 g/kg DM respectively. This shows that the variation in hemicellulose content may not be the contributing factor to this variation. Instead, it may be the method used to measure NDF and the timing of harvest. Comparatively, the perennial ryegrass hemicellulose had larger variation 207-257 g/kg DM yet low variation in NDF. These results suggest that ryegrass may be trying to achieve a consistent NDF, but this cannot be proven.

Similar to ADF, white clover has the lowest NDF, which is potentially associated with plant structure. The prostrate structure should not require additional structural components (hemicellulose) compared to other species. Hence the small difference in ADF and NDF. However, no studies in Table A3 presented both ADF and NDF measures. Red clover also had a low hemicellulose content (80-83 g/kg DM) from Moorby *et al*. (2004) and Vasiljević *et al*. (2011). The low hemicellulose content in both clovers suggests that there may be an interaction between legumes and hemicellulose content. This is likely due to the high digestibility of legumes compared to non-legumes.

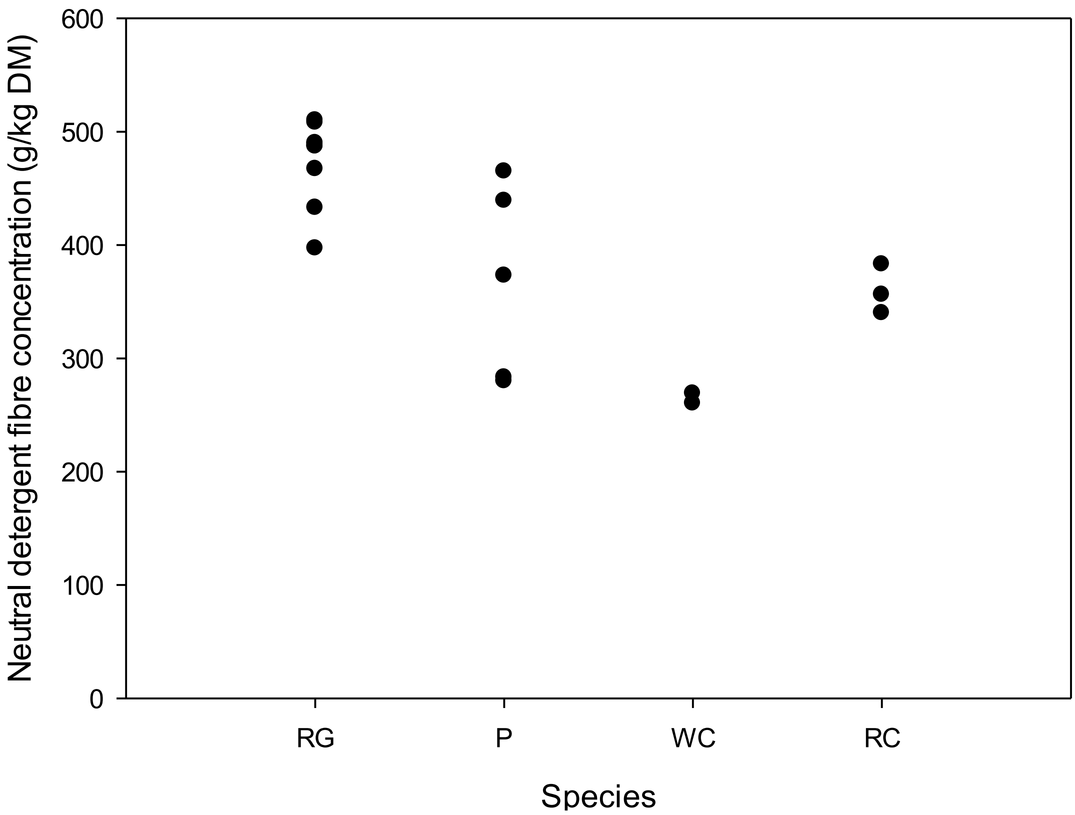

Figure 2.5: Neutral detergent fibre content (g/kg DM) of perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) summarised from Tables A3, A4, A5 and A6.

All the measurements of nutritive value interact with each other to provide an indication of the quality of the pasture. To fully understand the quality of the pasture, all these measurements and relationship/interactions between these must be understood. CP and ME are the main presented factors of nutritive value, yet the factors that influence the value that is returned from analysis tend to be neglected. The published measures of nutritive value in Tables A3 to A6 begin to show how the relationship between components interacts. Furthermore, the effect of cultivar could influence the measures of nutritive value depending on the breeding background of the plants. As the measures of nutritive value presented in Tables A3 to A6 were all from different cultivars which makes direct comparisons difficult.

Pastoral agriculture relies on the ability to find species that complement each other to optimise production. Perennial ryegrass, white clover, red clover and plantain can all be combined in a singular pasture sward. White clover had the highest CP concentration of the four species in the order of white clover 250 g/kg DM > red clover 219 g/kg DM > plantain 204 g/kg DM > perennial ryegrass 185 g/kg DM (Figure 2.2). The higher CP was expected in the legume species due to their ability to fix N. However, plantain cannot fix N, hence the greater crude protein content of plantain compared to perennial ryegrass could be a function of the timing of pasture cuts for analysis. Perennial ryegrass had a similar ME as plantain (10.8 compared to 10.6 MJ ME/kg DM) (Figure 2.3). The lower ME of plantain is likely to be associated with the 9.5 MJ ME/kg DM measured by Fulkerson *et al*. (2008). Red and white clover had higher ME than both perennial ryegrass and plantain, at 11.1 and 11.5 MJ ME/kg DM respectively. The combination of the lower NDF and higher ME of the clovers is associated with greater intake and preference by grazing animals (Penning *et al*., 1991).

Pasture mixtures have the potential to influence the interactions between species to maximise nutritive value. Perennial ryegrass and white clover are the common pasture species sown in a mixture in New Zealand. However, they are known to be nutritively limiting during summer. Litherland *et al*. (2002) showed a variation of the nutritive value of sheep and beef pastures in Waikato, Tararua, Canterbury and Southland regions. The highest ME pastures were found in spring and were highest in Southland in autumn (10.0 MJ ME/kg DM) and Tararua in spring (11.6 MJ ME/kg DM). Alternative pasture mixtures may have an additional pasture species to combine with the ryegrass and white clover or replace one of these species. Table 2.2 shows a comparison of the CP and ME of herb-clover and perennial ryegrass-white clover pastures. Both pasture mixtures contained legumes and non-legumes, therefore the interactions between the species will contribute to the differences in CP and ME.

Diversity effects have been observed when mixing species together which have differing characteristics (Harris, 1968). These diversity effects are most commonly measured as the increase in yield as a result of mixing grass with a legume. The potential for diversity effects to be observed in the form of nutritive value has not been identified. Neither has a comparison of nutritive value of individual species and the subsequent nutritive value a mixture including these species. As seen in Figure 2.3, there is little variation in the ME of the different species. Therefore, the opportunity for a diversity effect to be observed may be limited, but this is yet to be investigated.

From all the studies presented in Table 2.2, the herb mix had a higher ME than the perennial ryegrass-white clover pasture. However, there was little variation in the individual ME (Figure 2.3). Therefore, either the presence of chicory was having a positive effect on ME or the presence of clover may be proportionally higher than the ryegrass clover sward. Interactions between species and higher proportions of clover was likely to be the causing factor of the higher ME in the herb clover mixture. The proportions of species within the two swards may be a contributing factor to these differences, as well as variation between studies presented in Table 2.2. The relationship between OMD and ME may be the causing factor for the higher ME of the herb clover mixture. However, without this information being presented with the ME of the sward, it is not possible to make this assumption that this relationship is the causing factor of the ME differences between the two mixtures.

Mixtures have various underlying interactions which determine the nutritive value of the pasture. Mixing species together can have various outcomes depending on synergistic or antagonistic effects among species (Black *et al*., 2017). The outcome of mixing species is commonly observed in terms of yield variation. Nutritive value may show the same trends depending on interactions which may follow the same pattern as yield. Comparison with the individual ryegrass and white clover CP shows that mixing these two species resulted in the average CP being 25% (or 250 g/kg DM) from Table 2.2. Comparatively, the mid species mean from monocultures of ryegrass and white clover is

218 g/kg DM. Therefore, on average mixing these species together resulted in a yield increase and CP content increase compared to the monocultures. ME did not show the same relationship as CP when comparing the monocultures with the mixture. Average ME from Table 2.2 was 9.77 MJ ME/kg DM while the mean from the monocultures in Figure 2.3 was 11.15 MJ ME/kg DM. This may be a function of the lower ME ryegrass dominating the sward. The interactions among the four species in the herb clover mixture are difficult to extrapolate as there are four species involved. Therefore, the interactions are more complex than a two-species mixture as some species may be interaction more strongly than others. Proportion data could be beneficial in determining these relationships and how they influence the nutritive value of the mixture.

Table 2.2: Crude protein (CP) and metabolisable energy (ME) content of the herb and clover mix (herb mix); containing chicory, plantain, red clover and white clover compared to perennial ryegrass and white clover (RG/WC) across the seasons; early spring (September/October), late spring (November/December), summer (January/February), early autumn (March/April) and late autumn (April/May) in the Manawatu region, New Zealand (Cranston *et al*., 2015).

Season and study	CP (% DM)		ME (MJ ME/kg DM)	
	RG/WC	Herb mix	RG/WC	Herb mix
Early spring				
Kenyon *et al*., 2010	13	13	9.2	10.5
Hutton *et al*., 2011	12	15	10.6	10.8
Somasiri, 2014 *	25	20	10.4	11.4
Late spring				
Somasiri *et al*., 2015a *	16	23	10.2	11.2
Summer				
Kenyon *et al*., 2010	9	9	8.9	9.8
Hutton *et al*., 2011	9	12	9.5	9.9
Somasiri *et al*., 2015b *	15	18	9.8	10.9
Early autumn				
Golding *et al*., 2011	20	16	9.0	11.4
Late autumn				
Somasiri, 2014 *	24	23	10.4	11.3

* Studies were averaged over two years

2.5 Sowing methods

Sowing methods that separate species in alternate drill rows may influence the subsequent interactions between these species. Physical separation of species through alternate drill rows can reduce the competition between species during establishment. Similarly, temporal separation can allow for slow establishing species to establish before there is competition from other species. Hurst *et al*. (2000) investigated such sowing techniques with regards to their respective yield. The treatments were: 1 spring sown clover, 10 kg/ha perennial ryegrass direct-drilled in autumn; 2 timothy plus clovers; 3 and 4: clovers with timothy and 3.5 kg/ha or 8 kg/ha ryegrass in alternating rows. The alternative rows sowing method yielded greater than those which had ryegrass sown later or replaced by timothy. Treatments 3 and 4 yielded significantly greater than Treatments 1 and 2 at all dates except March 1999. However, in the final year of both Treatment 3 and 4, over 50% of the botanical composition of the sward was red and white clover and timothy. Therefore, alternate row sowing may promote the growth of slow establishing species with time. The influence of alternative sowing on the nutritive value of pasture has not been investigated to test whether the interactions between species remain similar to mixtures where species are not separated. Therefore, this may be an area for further research.

Table 2.3: Dry matter production (t DM/ha) from establishing dairy pastures over 16 months from sowing in November 1998. Treatments 1: spring sown clover, 10 kg/ha perennial ryegrass direct-drilled in autumn; 2: timothy plus clovers; 3 and 4: clovers with timothy and 3.5 kg/ha or 8 kg/ha ryegrass in alternating rows (Hurst *et al*., 2000).

Treatment	Jan 1999	Mar 1999	Oct 1999	Dec 1999	Jan 2000	Feb 2000	Mar 2000	Total (t/ha)
1	1.0	2.7	2.2	2.4	2.3	2.1	2.1	14.8
2	1.5	2.9	2.3	2.6	2.2	2.2	1.8	15.6
3	2.2	3.2	2.7	2.8	2.7	3.1	2.7	19.4
4	2.4	2.5	2.6	2.9	2.8	3.0	2.6	18.8
SEM	0.16	0.32	0.10	0.11	0.09	0.08	0.08	0.56

Diamond or square drilling refers to drilling the same area twice at a different angle to the first pass. Square drilling involves the second pass being at 90° to the first, while diamond drilling is at 30-40°. The diamond drilling pattern results in less competition on the young seedlings than the square drilling pattern due to orientation and interaction. Thom & Ritchie (1993) investigated the use of square and diamond drilling to establish species and whether there was an increase in yield as a result of this establishment method. Four treatments were used: two sowing rates and either drilled once or diamond drilled. The high sowing rate (22 kg/ha ryegrass or 30 kg/ha tall fescue (*Festuca arundinacea*)) yielded greater with the diamond drilled treatment (13,400 kg DM/ha compared to 11,933 kg DM/ha). While the low sowing rate (11 kg/ha ryegrass or 18 kg/ha tall fescue) yielded higher under the standard drilling treatment (12,008 kg DM/ha compared to 13,155 kg DM/ha). The lack of a significant difference among these treatments demonstrates that the use of double pass drilling does not increase grass production during its early development and highlights the ability to tiller as the low sowing rate treatments reached the same yields as the high sowing rates. Overall, research surrounding alternative sowing strategies is limited but the results from Thom & Ritchie (1993) suggest that there is no additional benefit from using double pass drilling. There could be the potential for further research to test whether separating species using this pattern could benefit slow establishing species. However, it may not be possible due to the recommended sowing rates. Overall, the current sowing strategy as single pass in 15-20 cm rows seems to be the most practical but does not limit yield compared to other double pass sowing techniques. Otherwise, to separate slow and fast establishing, sowing in alternative rows may benefit early production. However, this would require a drill capable of doing this.

To benefit slow establishing species without separation, lowering the sowing rate of the dominant or most competitive species may reduce competition during the establishment phase. The traditional sowing rate of white clover in a pasture mix is 2-4 kg/ha (Black *et al*., 2006). In contrast, perennial ryegrass can be up to 30 kg/ha (Thom *et al*., 2011) which can be unnecessarily high. Black *et al*. (2006) suggested 8-12 kg/ha of ryegrass was optimum for the successful establishment of white clover. By reducing the sowing rate of perennial ryegrass. Thus, reducing the competitive advantage of perennial ryegrass during the early phases of establishment. Subsequently allowing for greater light interception by white clover (Black *et al*., 2009) during early development. Therefore, there is the possibility to increase the proportion of white clover within the developed sward. Aside from the recommendations to lower the sowing rate of perennial ryegrass, there have been no advances in the research associated with the increase in the sowing rate of white clover. The manipulation of white clover sowing rate could benefit its establishment through a higher population. However, this may result in higher interspecies competition. The recommendations to decrease the sowing rate of perennial ryegrass when sown with white clover have struggled to reach the practical aspect of pasture establishment. Without sowing rate of perennial ryegrass being lowered, white clover will continue to be outcompeted during establishment. The ability of white clover to contribute its desired use to a pasture becomes questionable.

2.6 Quality analysis

Nutritive value analysis is conducted to establish the quality of the pasture. Analysis can be achieved through *in vitro* methods where the pasture is broken down within an animals' rumen to establish the composition of the pasture in terms of quality. Near infrared (NIR) spectroscopy involves passing light through the material of interest. Both methods can provide accurate results about the quality of the pasture. NIR uses the wavelengths 1200-2500 nm, outside of these wavelengths below 1200 nm; the absorption bands are so weak that quantitative measurements are difficult while above 2500 nm the bands are so strong that measurements are difficult (Norris, 1989).

NIR has the ability to be calibrated against wet chemistry for the pasture type. An unknown pasture can then be tested against the known calibration to measure its quality. Calibration is the mathematical process required to relate NIR optical measurements to the properties used to define the nutritional quality of a forage. Enabling an unknown sample to be measured against a reference population of similar composition to interpret the nutritive value of the sample. Calibration must therefore include all the possible sources of variation that are likely to be found in the unknown sample (Deaville & Finn, 2000). At least 50 samples and as many as 200 samples of the reference population are required for wet chemistry calibration in order to produce robust calibrations (Deaville & Finn, 2000). Many studies which use NIR to interpret nutritive value calibrate their optical measurements against their sample population to attempt to reduce error in the results.

Sturludóttir *et al*. (2013) calibrated using 55 samples with the highest relative significant spectra from the 360 samples taken during the experiment. A further 15 samples were used for validation. These samples were analysed for ADF, NDF, in vitro true digestibility (IVTD) and N concentration. CP was calculated by multiplying N concentration by 6.25. Other studies such as Nobilly *et al*. (2013) used preset calibrations for samples. Calibrating NIR to the specific pasture type being used is beneficial to ensuring the result of NIR are consistent for the mixtures. However, robust calibrations which have been done for pastures in general should produce similar results. Maintaining a consistent form of measurement for nutritive value will result in consistent results. However, there will be variation between the results when comparing the different calibration methods.

2.7 Mixture experiments

Mixture experiments are used to investigate the interaction among components and how the proportions of components influences the expression of the mixture. Mixture experiments can be applied to a range of disciplines, from chemical mixtures to pastures. Cornell (2002) showed that a basic mixture design could be carried out based on simplex lattice or simplex centroid designs depending on the number of components to be included in the mixture. Lattices define the components to be included within each mixture based on the surface area of the lattice. These mixtures can include either three or four components at different rates defined by the simplex co-ordinate system (Cornell, 2002). Simplex centroid designs are similar to that of lattice designs but have the ability to include mixtures which are within the three-dimensional space. This includes the centroid mixture where all four components are represented equally in the mixture and other mixtures where all four species are included. Mixtures produced based on the principles shown by Cornell (2002) allow for the mixture design to minimise the influence of confounding. As each component can be included in several different mixtures, with all components having the same mixtures defined. Therefore, the expression of each mixture with the different components in the same proportions can be compared.

Previous pasture mixture experiments have not always minimised the potential for confounding through their mixture design. These mixtures tend to include up to seven species to test for diversity effects of a mixture, but do not include monocultures of each species. Lack of inclusion of all the monocultures does not quantify whether the mixture performs better than all the monocultures at the same site. These mixtures are usually compared based on animal production with a ryegrass-white clover pasture of varying ages (Woodward *et al*., 2013). Nobilly *et al*. (2013) compared the production of simple (three different grasses each sown with white clover) and diverse pastures (the same grass clover mixtures with up to four additional species) to test whether diverse pastures offered better production. The potential confounding within these studies which do not include the monocultures for reference cannot show the true benefit or lack of benefit for the proposed mixture. Comparatively, studies which include the monocultures and various combinations of species can provide additional information on the benefits of mixing species. Kirwan *et al*. (2007) used a simplex centroid design at each site (15 sites in total) to define four monocultures and 11 mixtures. These mixtures were then sown at two levels of abundance. This mixture design was repeated in subsequent years by Sturludóttir *et al*. (2013), Suter *et al*. (2015) and Connolly *et al*. (2009). These mixture designs allow for the majority of mixtures to be investigated to test how the species interact with each other and the subsequent impact on the aspect of interest.

2.8 Conclusions

- Pasture mixture formulation in the past has had little scientific basis behind the selling point of commercial mixtures.
- Animal production tends to be related to higher nutritive value rather than the species present.
- Many previous nutritive value experiments have shown the nutritive value of an individual species with comparisons against ryegrass-white clover pastures.
- Sowing method can influence competition between species during establishment, the subsequent influence on nutritive value has not been investigated.
- Analysis of nutritive value is commonly done through NIR calibrated to the specific pasture.
- Identifying the true effect of mixtures require the ability to identify the species effect before the diversity effect.

From the review of the literature, it can be seen that there is a limitation to the ability to measure nutritive value in pasture mixtures. Additionally, it hasn’t been shown whether nutritive value shows the same diversity effects, synergism or antagonism as yield when mixing species with different characteristics. Therefore, the following sections of this dissertation will aim to establish the nutritive value of tetraploid perennial ryegrass, plantain, white clover and red clover monocultures and how mixing these species together in various proportions influences nutritive value.

3 MATERIALS AND METHODS

3.1 Experimental design

Four common pasture species (two non-legumes and two legumes) and a single commercially available cultivar of each species, were chosen for the mixture study: perennial ryegrass cv. Base, plantain cv. Tonic, white clover cv. Apex and red clover cv. Grasslands Sensation. Stewart *et al*. (2014) described the basic characteristics of each species and cultivar. 'Base' is a tetraploid perennial ryegrass with a very late heading date (+22 days). 'Tonic' is winter active and in 2014 was the only certified cultivar of plantain available in New Zealand. 'Apex' is a medium-small leaved white clover plant. 'Grasslands Sensation' red clover has a medium level of oestrogen, is early flowering and usually persists three to five years. The perennial ryegrass, plantain and red clover had semi-erect growth habits, producing leaves from several tillers (ryegrass) or crown shoots (plantain and red clover) on a plant. By comparison, white clover had a prostrate growth habit and produces leaves and roots from nodes along stolons (horizontal stolons) which grow across the soil surface. The perennial ryegrass and white clover have fibrous root systems, plantain has a stronger, branched root system, and red clover produces a taproot below the crow shoots.

Nineteen seed mixtures varying in species richness from one to four species and in species relative abundance were created using a simple centroid design (Cornell, 2002) in the statistical software Minitab 17®. These were: four monocultures, six two-species mixtures (½ of each of two species), four three-species mixtures (⅓ of each of three species), one four-species centroid mix with each species equally represented (¼ of each species) and four four-species mixtures dominated in turn by each species (⅝ of one species and ⅛ of each of the other species). The proportion of the species in a mixture summed to one. The 19 mixtures in the four component simplex centroid design, illustrated by the tetrahedron diagram in Figure 3.1.

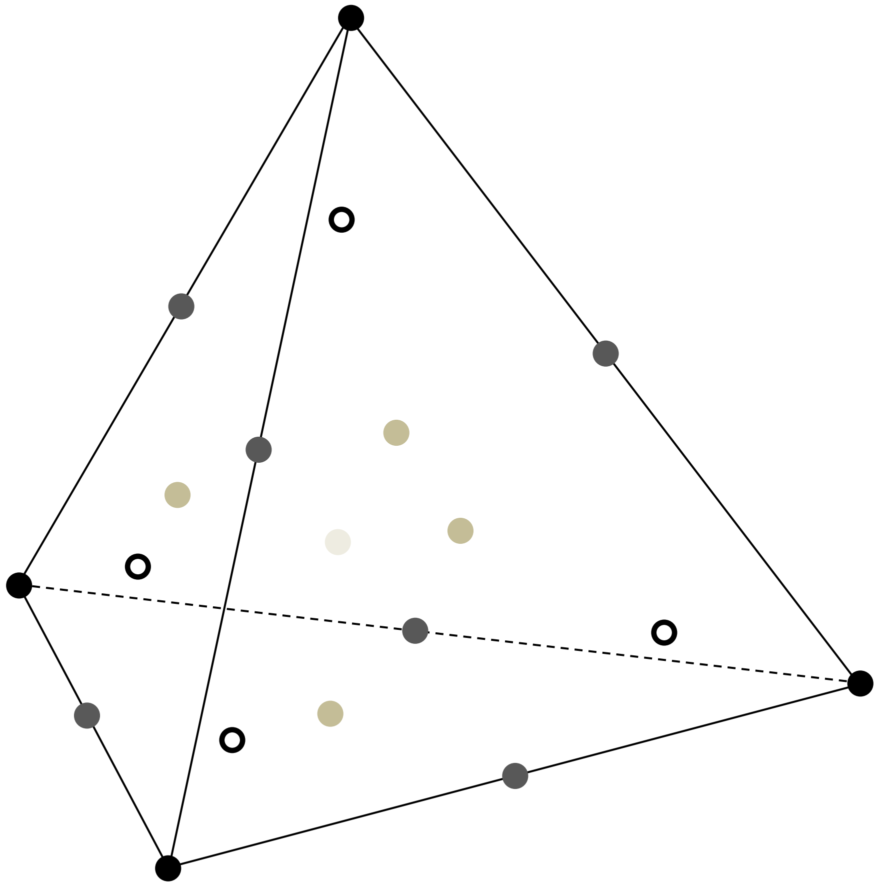

Figure 3.1: Four-species simplex centroid design with four monocultures (black circles), six two-species mixtures (½ of each of two species; dark grey), four three-species mixtures (⅓ of each of three species; medium grey), a four-species centroid mixture (¼ of each species; light grey) and four four-species mixtures dominated in turn by one species (⅝ of one species and ⅛ of each other species; white) (Black *et al.*, 2017).

A subset of the experiment design was repeated with species sown in alternate drill rows in the mixture, compared with the standard sowing method of species sown together in the same drill rows. Four of the nineteen mixtures were repeated with the second sowing method: three two-species mixtures (½ of each of two species) and one three species mixture (⅓ of each of three species) (Plate 1) of ryegrass, plantain and white clover. Red clover was excluded from this sowing method treatment because it was only possible to drill two and three species in alternative rows, not four species. The data from these treatments were used to test whether there was an effect of spatial separation of species on the species identity and interaction effects. In other words, are the species and mixture effects consistent across a standard sowing method and an alternative sowing method?

The 23 mixture sowing methods combinations were allocated to plots according to a randomized complete block design with four replicates, giving 92 plots in total. The plot size was 2.1 m x 6 m and each replicate was 48.3 m x 6 m. A 2 m space sown with turfgrass was allowed between each replicate block and around the perimeter of the experiment, so the total area of the experiment site was 52.3 m x 34 m.

Plate 1: Alternate row sowing method treatment of ryegrass, plantain and white clover.

3.2 Experimental site and pasture establishment

The mixture experiment was carried out in paddock H8 at the Horticultural Research Area at Lincoln University, Canterbury, New Zealand (43°38’53.14”S 172°27’11.60”E and 10 m above sea level) in a Templeton silt loam soil. Templeton soils are free draining with at least 0.46 m of fine greywacke from ancient flood waters of the Waimakariri River overlying gravels (Cox, 1978). A common Templeton silt loam soil profile has 0.23 m of topsoil on top of stones and gravels packed with sand. The site had previously been in lucerne (*Medicago sativa*) in 2011 and 2012, oilseed rape (*Brassica napus*) in 2013 and forage oats (*Avena sativa*) in 2014. The site was then ploughed and tilled in November 2014, sprayed with Roundup ULTRA® MAX (570 g/L glyphosate at 3 L/ha) in February 2015 and irrigated (60 mm) and then tilled into a seedbed in March 2015.

The 23 mixture-sowing method combinations were sown on the 26th of March 2015 at one overall level of abundance based on a seed count of 833 seeds/m^2. This sowing rate was equivalent to 30 kg/ha of 'Base' perennial ryegrass (Table 3.1) and was within the range of commercial sowing rates recommended by the seed industry for pasture mixtures in New Zealand (Stewart *et al*., 2014). The average thousand seed weights were 3.6, 2.7, 0.9 and 2.1 g, and germination percentages were 93, 99, 91 and 88% for ryegrass, plantain, white clover and red clover, respectively. The white clover seed was coated with AGRICOTE Clover seed treatment, which is a lime-based coating with molybdenum and provides protection against root nematodes and damping off. The other species were not treated. The sowing rate was not adjusted for germination percentage. The mixtures were sown using a Flexiseeder precision plot drill with 14 coulters spaced 0.15 m apart. The drill was capable of sowing up to three species in alternate rows. The sowing depth was 10-15 mm.

Table 3.1 Sowing rates in kilograms of seed per hectare of 19 seed mixture varying in proportions of perennial ryegrass (x_1), plantain (x_2), white clover (x_3) and red clover (x_4) at an overall abundance of 833 seeds/m^2.

		Components				
	Mixture	x_1	x_2	x_3	x_4	Total
Monoculture	1	30	0	0	0	30
	2	0	22.5	0	0	22.5
	3	0	0	7.5	0	7.5
	4	0	0	0	17.5	17.5
Two-species mixture	5	15	11.25	0	0	25.25
	6	15	0	3.75	0	18.75
	7	15	0	0	8.75	23.75
	8	0	11.25	3.75	0	15
	9	0	11.25	0	8.75	20
	10	0	0	3.75	8.75	12.5
Three-species mixture	11	10	7.5	2.5	0	20
	12	10	7.5	0	5.83	23.33
	13	10	0	2.5	5.83	18.33
	14	0	7.5	2.5	5.83	15.83
Four-species mixture	15	7.5	5.63	1.88	4.38	19.38
	16	18.75	2.82	0.94	2.19	24.69
	17	3.75	14.06	0.94	2.19	20.94
	18	3.75	2.82	4.69	2.19	13.44
	19	3.75	2.82	0.94	10.94	18.44

3.3 Management

All plots were grazed by sheep (except in August 2015 where the plots were mown to prevent treading damage) and mowed after grazing to an even residual height of approximately 40 mm. Harvest dates and regrowth durations are presented in Table 3.2

Table 3.2: Grazing management of the plots for the duration of the current and previous year for the harvests used for NIR analysis for the duration of the experiment.

Year	Harvest	Date sampled	Sheep in	Sheep out	Date mowed	Days growth
2	1	4/08/15	Not grazed	Not grazed	16/08/15	143
2	2	21/09/15	22/09/15	24/09/15	25/09/15	37
2	3	23/10/15	27/10/15	29/10/15	30/10/15	32
2	4	30/11/15	1/12/15	3/12/15	3/12/15	32
2	5	6/01/16	7/01/16	10/01/16	22/02/16	36
2	6	15/02/16	16/02/16	20/02/16	22/02/16	36
2	7	31/03/16	1/04/16	4/04/16	5/04/16	39
2	8	23/05/16	27/05/16	30/05/16	30/05/16	52
3	1	2/08/16	2/08/16	3/08/16	5/08/16	64
3	2	22/09/16	26/09/16	30/09/16	30/09/16	52
3	3	31/10/16	1/11/16	7/11/16	7/11/16	32
3	4	9/12/16	9/12/16	13/12/16	13/12/16	32
3	5	13/01/17	16/01/17	19/01/17	19/01/17	32
3	6	17/02/17	17/02/17	21/02/17	21/02/17	29
3	7	30/03/17	31/03/17	5/04/17	7/04/17	38
3	8	26/05/17	26/05/17	30/05/17	30/05/17	49

Soil analysis on 4 May 2015 showed pH 5.7, Olsen P 13 mg/L, Ca 7.3 me/100 g, Mg 0.84 me/100 g, K 0.32 me/100 g, Na 0.17 me/100 g and sulphate S 13 mg/kg. Superphosphate (9% P, 11% S) was applied at 500 kg/ha on 30 September 2015 and 480 kg/ha on 12 October 2016. Irrigation was applied at a total rate of 360 mm every 3-5 weeks from 30 October to 6 April 2016 and 260 mm from 9 November 2016 to 15 February 2017 to maintain soil moisture above a critical limit of 24% (Black & Murdoch, 2013).

T-max™ (30 g/L aminopyralid at 2 L/ha) was applied to remove volunteer white clover from plots with no sown clover. Ryegrass monoculture plots were sprayed on 10 March 2017 and the plantain monocultures and the ryegrass-plantain plots were sprayed on 5 May 2017. All plots were sprayed with Dew™ 600 (600 g/L diazinon at 4 L/ha) to control grass grub (*Costelytra zealandica*) on 10 May 2017.

3.4 Measurement

Before each harvest, one representative sample from each plot was clipped to 10-20 mm residual using electric hand clippers. All treatments were harvested as one metre long of two drill rows (0.3 m^2), expect for the three-species sown in alternative rows where the harvested area was extended to three drill rows (0.45 m^2). Once cut, samples were stored in a chiller at 3°C for a maximum of 5 days. A representative grab sample was taken from each cut sample, separated into the sown species, volunteer white clover and weeds. The main weed species included annual poa (*Poa annua*) couch grass (*Elymus repens*), dock (*Rumex obtusifolius*), plantain, perennial ryegrass and dandelion (*Taraxacum officinale*). Ryegrass, plantain and white clover weeds could not be differentiated from their sown counterparts, therefore were not separated unless the species was not sown in the pasture mixture. After harvest, a 0.2 m^2 quadrat per monoculture plot and a centroid plot were clipped to 10-20 mm. All plant material was dried in a fan forced oven at 60°C for at least 48 hours. Once dry, the weights of all components were recorded to calculate the yield of each pasture mixture. Yield was calculated as the pre-harvest herbage mass less the previous residual herbage mass.

Nutritive value of the harvested herbage was measured for each plot for harvest 5 and 7 in year 2 and all harvests for year 3. Dried unseparated samples were ground to pass through a 1 mm sieve using a Retsch ZM 200 grinder (Retsch, Germany) before being collected. Samples were scanned using near infrared spectroscopy (NIRS; FOSS NIRSystems 5000, FOSS NIRSystems Inc., Laurel, MD, USA) at the Lincoln University Analytical Laboratory (Plate 2) to determine CP, ADF, NDF and digestible organic matter (DOMD). Metabolisable energy (ME) was calculated as: ME (MJ/kg DM) = 0.016*DOMD (McDonald *et al.*, 2002), where DOMD is in grams per kilogram of DM. Calibration was based on a pasture where 250 samples for each measure were used to provide a wide reference population for the measures: DMD, OMD, DOMD and protein. No further calibration was carried out relative to these specific pasture mixtures.

Annual weighted mean was calculated as:

$$QV_i = \sum_{j=1}^{h} \left(QM_{ij} \times y_j\right) \div \sum_{j=1}^{h} y_j$$

where QM_{ij} is the nutritive value *i* in harvest *j*, and y_j is the dry matter yield of harvest *j*, and QV_i is the annual measure for nutritive value *i* (Sturludóttir *et al*., 2013).

Components of nutritive value analysed from each harvest were: ME concentration, ADF concentration, NDF concentration, CP concentration, ME yield (ME concentration × yield) and CP yield (CP concentration × yield). As well as annual yield, annual ME yield, annual CP yield which were summed for each plot and annual weighted means for ME concentration, ADF concentration, NDF concentration and CP concentration.

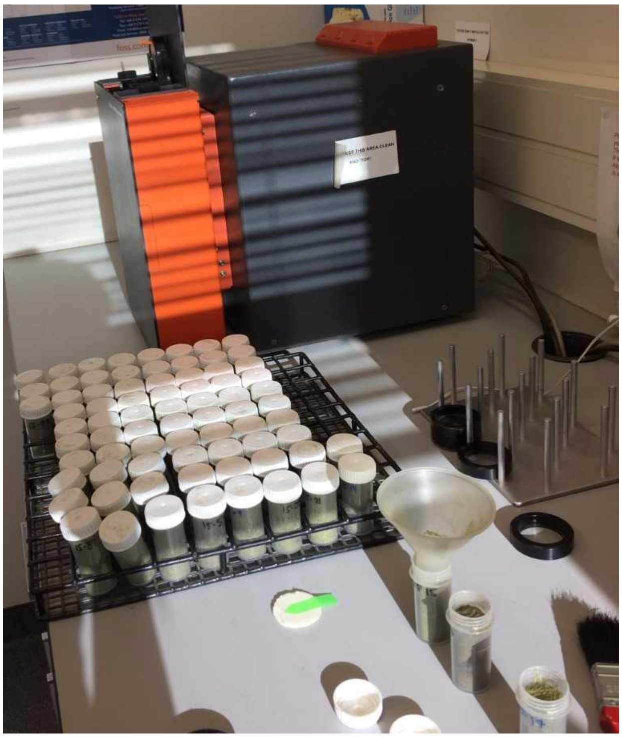

Plate 2: NIR analysis at the Lincoln University Analytical Lab.

3.5 Statistical analysis

Nutritive value was analysed using the mixture regression method in Minitab® 17 statistical software where a special cubic model was fitted to the data for each harvest followed by an annual weighted mean (concentrations) and annual yield for the 2016/17 production year. The model used can quantify separate two-species, three-species and four-species interactions in the mixtures. It had the general form:

$$\hat{y} = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{14}x_1x_4 + \beta_{23}x_2x_3 + \beta_{24}x_2x_4 + \beta_{34}x_3x_4 + \beta_{123}x_1x_2x_3 + \beta_{124}x_1x_2x_4 + \beta_{134}x_1x_3x_4 + \beta_{234}x_2x_3x_4 + \beta_{1234}x_1x_2x_3x_4 + \varepsilon \quad \text{(Model 1)}$$

where $\hat{y}$ is the predicted response from a mixture, x_1, x_2, x_3 and x_4 are the sown proportions of ryegrass, plantain, white clover and red clover respectively. β_1 to β_4 are estimates of the response of the monocultures. β_{12} to β_{34} represent the interaction effects for the combination of two species. β_{123} to β_{234} are the additional interaction effects for the combination of three species. β_{1234} is the additional interaction effect for the combination of four species. ε is a random error term assumed to be normally and independently distributed with mean of zero and constant variance.

The analysis of variance tested whether the estimates were significantly different ($p<0.05$) to zero. The significant estimates where re-analysed to give the coefficients which would be included within each model. The models fitted to the annual weighted means and annual yields were used to construct contour plots.

The response optimisation function in Minitab® 17 searches the entire tetrahedron design space (Figure 3.1) to define the optimal seed mixture based on the target values for annual yield, annual ME and CP yield, and annual weighted means for ME, ADF, NDF and CP concentrations (Table 3.3).

Table 3.3: Target values for each of the measured nutritive value constituents used in the response optimiser function in Minitab® 17 to produce the optimal seed mix.

	Lower	Target	Upper
Yield (kg DM/ha/yr)	3600	15000	16000
ME (MJ ME/kg DM)	10.0	12.0	13.0
ADF (g/kg DM)	250	270	280
NDF (g/kg DM)	300	380	420
CP (g/kg DM)	160	200	230
ME yield (GJ/ha/yr)	120	140	200
CP yield (kg/ha/yr)	2700	2800	3400

Alternate row treatments were analysed using the mixture regression function in Minitab® 17. A quadratic model was fitted to the alternate row data and the data where the species were sown together for the monocultures of ryegrass, plantain and white clover and the mixtures of ryegrass-plantain, ryegrass-white clover, plantain-white clover and ryegrass-plantain-white clover. The monoculture data had to be repeated as if it were in alternate rows to produce a balanced design. The model had the general form:

$\hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \beta_{12} x_1 x_2 {*}\text{treat} + \beta_{13} x_1 x_3 {*}\text{treat} + \beta_{23} x_2 x_3 {*}\text{treat} + \beta_{123} x_1 x_2 x_3 {*}\text{treat} + \varepsilon$

where $\hat{y}$ is the predicted response from a mixture, x_1, x_2 and x_3 are the sown proportions of ryegrass, plantain and white clover respectively. β_1 to β_3 are estimates of the response of the monocultures. β_{12} to β_{23} represent the interaction effects for the combination of two species. β_{123} to β_{123} are the additional interaction effects of the three species. The treat term represents the interaction effect of sowing methods, used as a process variable in the analysis. The treatments were coded as -1 for species sown together and 1 for species sown in alternative rows.

3.6 Climate

Climate data on total monthly rainfall and average monthly temperature (Figure 3.2) were acquired from the Broadfields weather station, near Lincoln township (approximately 1.9 km from the site). Lincoln-Broadfield, network number 17603 H32645, located at latitude -43.62622 and longitude 172.4704 at 18 m above sea level. The observing authorities were National Institute of Water and Atmospheric Research Ltd (NIWA) and Plant & Food Research.

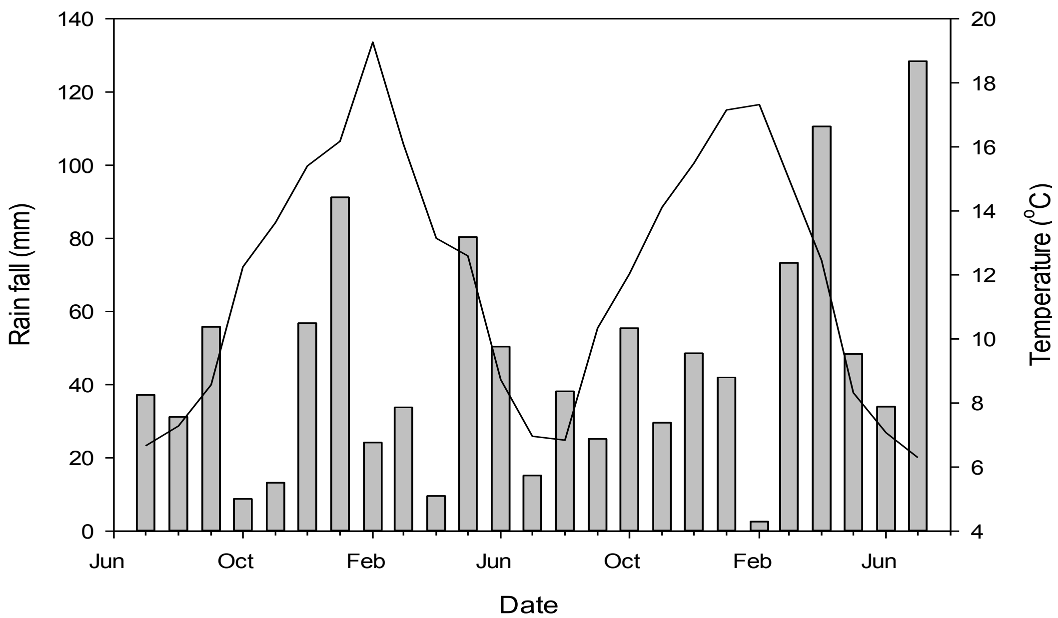

Figure 3.2: Monthly rainfall and average monthly temperature at Lincoln-Broadfield weather monitoring station from 1/07/15 to 31/07/17.

4 RESULTS

4.1 Optimal mixture

A mixture containing ryegrass, white clover and red clover with the proportions of 0.43 ryegrass, 0.20 white clover and 0.37 red clover was presented as the optimal mixture which maximises yield, ME yield, CP yield, CP, ME while minimising ADF and NDF of a three-year-old pasture. The potential composition of this pasture can be seen in Plate 3. The species proportions of the optimal seed mixture were equivalent to the seed rates of 12.90 kg/ha of ryegrass, 1.50 kg/ha of white clover and 6.48 kg/ha of red clover with a total sowing rate of 20.88 kg/ha. This mixture was predicted to have an annual yield of 14,250 kg DM/ha and have a CP concentration of 195 g/kg DM, ME 11.2 MJ ME/kg DM, ADF 264 g/kg DM and NDF 380 g/kg DM and annual ME yield of 160 GJ ME/ha and annual CP yield of 2,806 kg/ha. The nutritive value attributes of this optimal mixture can be predicted using the following models for the annual yield, ME yield and CP yield and annual weighted means for ME, CP, ADF and NDF. Plus, predictions for each of these properties for all the harvest dates.

Plate 3: Ryegrass, white clover and red clover pasture mixture.

4.2 Nutritive value models

Annual ME yield showed upward strong curves on the ryegrass-red clover and plantain-red clover axes (Figure 4.1). This suggested that a seed mixture of ryegrass-red clover, plantain-red clover or a combination of ryegrass, plantain and red clover would result in the greatest annual ME yield (Plate 4).

The model that generated the contour plots for annual ME yield is:

Annual ME yield (GJ ME/ha) = $112.87x_1 + 81.84x_2 + 113.16x_3 + 132.04x_4 + 105.89x_1x_3 + 231.81x_1x_4 + 147.44x_2x_3 + 257.73x_2x_4$ (Model 2)

where the proportion of species sown in the sward are represented as: x_1 is ryegrass, x_2 is plantain, x_3 is white clover and x_4 is red clover.

The model had adequate fit (R^2 = 63.58%, R^2 adjusted = 59.84%). The quadratic terms RG*P and WC*RC, the three species terms (RG*P*WC, RG*P*RC, RG*WC*RC, P*WC*RC) and the four-species term (RG*P*WC*RC) were insignificant (p>0.05) so they were removed from the model.

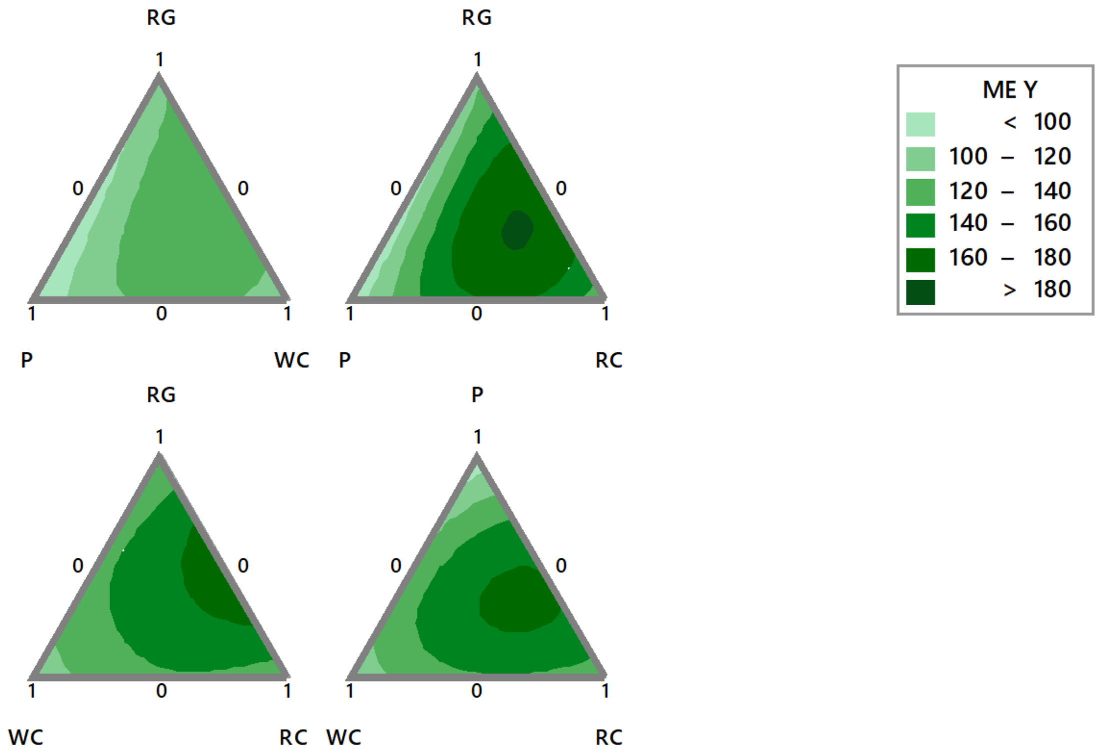

Figure 4.1: Contour plots of annual metabolisable energy (ME) yield (GJ ME/ha) predicted from Model 2 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.1) and coefficients (Table 4.1; Table B1) showed that the monoculture ME yields were greatest for red clover and least for plantain: 134.04 red clover > 113.16 white clover > 112.87 ryegrass > 81.84 plantain GJ ME/ha. This pattern was not consistent across all seasons measured (Table 4.1) where red clover had the lowest ME yield in August, highest in September and December, second highest in October, February and March and second lowest in May. Comparatively, plantain had the lowest ME yield in September, January and March, second lowest in October, December, February and second highest in May (Table 4.1). There were also differences among the pairwise interactions. Both ryegrass and plantain interacted more strongly with red clover than white clover, as indicated by the higher surfaces (darker colours) in Figure 4.1 and the coefficients in Table 4.1. The interactions of white clover with ryegrass and plantain was significant in the annual ME yield, but these interactions were only significant in January harvest. There was an additional interaction of white clover-red clover in January and February.

Plate 4: Ryegrass, plantain and red clover pasture mixture.

Table 4.1: Coefficients used in Model 2 from the mixture analysis for metabolisable energy (ME) yield (GJ ME/ha) per harvest and annual which were significant ($p<0.05$) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B1 shows all coefficients from analysis.

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	10.49	11.19	24.00	18.11	20.31	10.01	12.65	11.77	112.87
P	5.71	7.21	16.62	19.59	14.69	11.20	11.71	6.11	81.84
WC	5.45	14.31	16.19	21.32	28.99	17.68	17.74	0.66	113.16
RC	4.29	16.42	19.63	26.64	30.61	14.31	13.29	3.68	132.04
RG*P	-	-	-	-	-	-	-	-	-
RG*WC	-	-	-	-	33.32	-	-	-	105.89
RG*RC	-	-	-	61.10	57.15	47.90	45.32	-	231.81
P*WC	-	-	-	-	52.59	-	-	-	147.44
P*RC	-	32.42	-	41.64	65.95	45.01	49.61	-	257.73
WC*RC	-	-	-	-	31.62	23.31	-	-	-
RG*P*WC	-	-	-	-	-	-	-	-	-
RG*P*RC	-	-	-	-	-	-	-	-	-
RG*WC*RC	-	-	-	-	-	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual CP yield shows a slight upward curve on the ryegrass-red clover and plantain-red clover axes (Figure 4.2), suggesting the optimal mixture could be a mixture of ryegrass, plantain and red clover would result in the greatest annual CP yield (Plate 4). The model fitted to the contour plots for annual CP yield is:

Annual CP yield (g/ha) = $1407x_1 + 1223x_2 + 2411x_3 + 2680x_4 + 4874x_1x_4 + 4396x_2x_4$

(Model 3)

The model had adequate fit (R^2 = 69.50% and R^2 adjusted = 67.32%). The quadratic terms RG*P, RG*WC, P*WC and WC*RC, the three species terms and the four-species terms were insignificant ($p>0.05$) so were removed from the model.

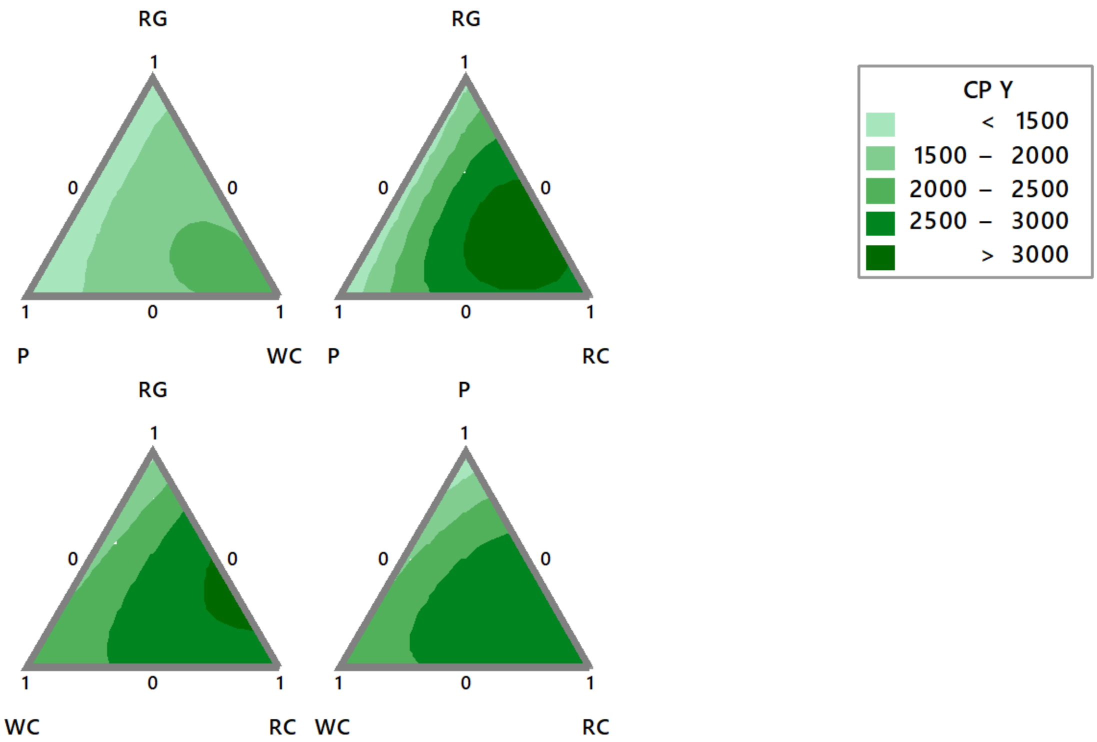

Figure 4.2: Contour plots of annual crude protein (CP) yield (kg/ha/yr) predicted from Model 3 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.2) and coefficients (Table 4.2; Table B2) showed that monoculture CP yields were greatest for red clover and least for plantain: 2,680 red clover > 2,411 white clover > 1,407 ryegrass > 1,223 plantain kg/ha/yr. This trend was not consistent throughout the entire production season, red clover had the highest CP yield in September, October and December, second highest in January, February, March and May and second lowest in August. While plantain was consistently the lowest, except in September, December and May. There were also differences among pairwise interactions, where ryegrass and plantain interacted more strongly with red clover than white clover based on the slope of the contour plots (Figure 4.2) and the coefficients (Table 4.2). These interactions of ryegrass and plantain with red clover were significant in the annual CP yield and in four harvests throughout the year. There was an additional interaction of white clover and red clover in January. Ryegrass and plantain interacted more strongly with red clover than white clover based on the slope of the contour plots and the significant coefficients.

Table 4.2: Coefficients used in Model 3 from the mixture analysis for crude protein (CP) yield (kg/ha/yr) per harvest which were significant (p<0.05) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B2 shows all coefficients from analysis

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	119.1	118.2	305.9	194.8	226.0	187.3	164.9	152.8	1407.0
P	71.4	141.0	253.8	255.4	206.3	160.1	143.8	75.1	1223.0
WC	83.7	276.4	325.7	375.8	579.4	336.5	342.0	10.1	2411.0
RC	81.9	409.1	445.0	557.4	579.0	324.2	291.0	86.5	2680.0
RG*P	-	-	-	-	-	-	-	-	-
RG*WC	-	-	-	-	-	-	-	-	-
RG*RC	-	-	-	1235.0	1089.8	837.7	978.0	-	4874.0
P*WC	-	-	-	-	-	-	-	-	-
P*RC	-	-	-	777.2	1191.1	677.7	803.0	-	4396.0
WC*RC	-	-	-	-	636.8	-	-	-	-
RG*P*WC	-	-	-	-	-	-	-	-	-
RG*P*RC	-	-	-	-	-	-	-	-	-
RG*WC*RC	-	-	-	-	-	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual weighted ME shows an incline in ME towards ryegrass (Figure 4.3). No other species had the same high ME as ryegrass, suggesting that the optimal pasture mixture which maximises ME would be a ryegrass monoculture.

The model fitted to the contour plots for annual weighted ME is:

ME (MJ ME/kg DM) = $12.16x_1 + 10.98x_2 + 11.44x_3 + 10.84x_4 - 1.82x_1x_4$ (Model 4)

The model had an adequate fit (R^2 = 71.01% and R^2 adjusted = 69.38%). The quadratic terms RG*P, RG*WC, P*WC, P*RC and WC*RC, the three species terms and the four-species term were all insignificant (p>0.05) so were removed from the model.

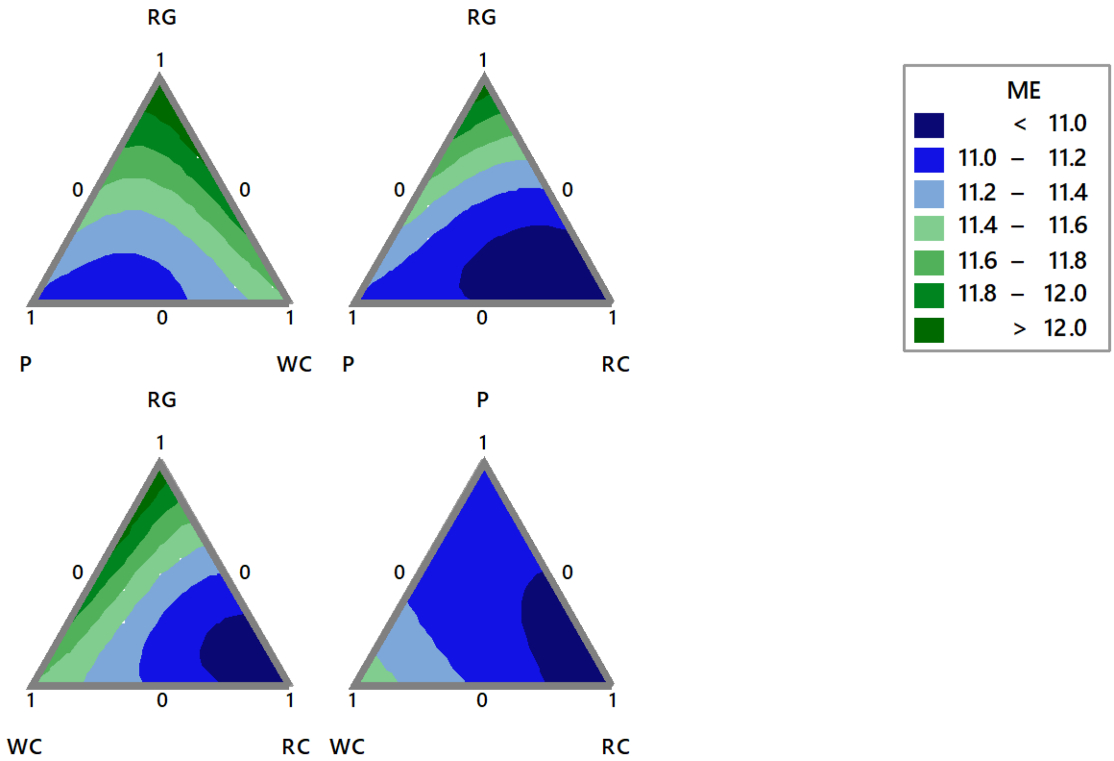

Figure 4.3: Contour plots for annual weighted metabolisable energy (ME) concentration (MJ ME/kg DM) predicted from Model 4 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.3) and coefficients (Table 4.3; Table B3) shows that ryegrass has the greatest ME while red clover has the lowest: 12.16 ryegrass > 11.44 white clover > 10.98 plantain > 10.84 red clover MJ ME/kg DM. Ryegrass consistently had the highest ME throughout the year from each harvest, except for being the second highest in September. Comparatively, red clover had the lowest ME for every harvest during the year except for December and January where it was second lowest. There were additional differences among pairwise interactions. Ryegrass interacted most strongly with white clover indicated by the slope of the contour plot (Figure 4.3), however, the negative influence of red clover is more notable based on the coefficients of the annual weighted mean (Table 4.3). The interactions between species varied throughout harvests, with interactions between ryegrass-white clover, ryegrass-red clover, plantain-white clover, white clover-red clover and three species interactions of ryegrass-white clover-red clover. These interactions were not consistent throughout the production season.

Table 4.3: Coefficients used in Model 4 from a mixture analysis for metabolisable energy (ME) (MJ ME/kg DM) per harvest which are significant ($p<0.05$) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B3 shows all coefficients from analysis.

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	12.55	12.03	12.44	11.81	12.51	11.21	11.54	12.73	12.16
P	12.08	11.27	11.52	10.56	10.52	10.81	11.25	12.54	10.98
WC	11.08	12.13	12.13	11.61	11.70	10.93	11.35	12.07	11.44
RC	11.06	11.17	11.26	10.83	10.73	10.70	10.61	11.11	10.84
RG*P	-	-	-	-	-	-	-	-	-
RG*WC	4.35	-	-	-1.52	-	-	-	-	-
RG*RC	-	-	-	2.64	-2.41	-	-	-	-1.82
P*WC	2.58	-	-	-1.51	-2.12	-	-	-	-
P*RC	-	-	-	-	-	-	-	-	-
WC*RC	-	-	-	-1.93	-	-	-	-	-
RG*P*WC	-	-	-	-	-	-	-	-	-
RG*P*RC	-	-	-	-	-	-	-	-	-
RG*WC*RC	21.69	-12.87	-	-	-13.24	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual weighted ADF showed a strong upward curve of increasing ADF on the axis between ryegrass and red clover, while plantain and white clover had low ADF as monocultures, when mixed together there is an increase in ADF (Figure 4.4). This suggested that the optimal pasture mixture to minimise ADF would consist of either a plantain or white clover monoculture or a mixture of the two species.

The model fitted to the contour plots for annual weighted ADF is:

ADF (g/kg DM) = $255.9x_1 + 250.3x_2 + 248.4x_2 + 254.1x_4 + 46.5x_1x_4 + 31.2x_2x_3$ (Model 5)

The model had a poor fit (R^2 = 25.68%, R^2 adjusted = 20.37%). The quadratic terms RG*P, RG*WC, P*RC and WC*RC, three species terms and four-species term were insignificant ($p>0.05$) so were removed from the model.

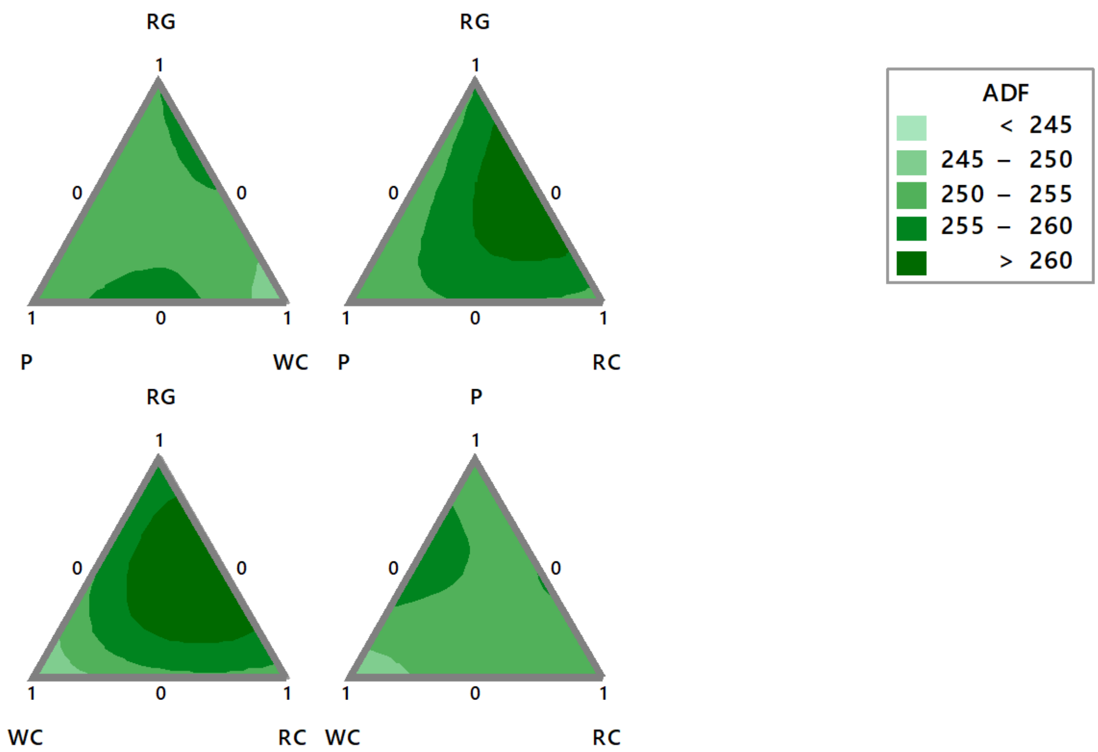

Figure 4.4: Contour plots of annual weighted mean of acid detergent fibre (ADF) concentration (g/kg DM) from Model 5 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.4) and coefficients (Table 4.4; Table B4) indicated that ADF concentration for monocultures was greatest for ryegrass and least for white clover: 255.9 ryegrass > 255.0 red clover > 250.3 plantain > 248.4 white clover. This order of ADF concentrations in the monocultures did not remain consistent throughout the harvests. However, ryegrass did have the highest ADF concentrations apart from January and February where it was second lowest (Table 4.4). White clover did not remain consistently the lowest ADF concentration, it had the highest ADF in February. Additional pairwise comparisons observed in the annual weighted mean showed that ryegrass interacted more strongly with red clover and plantain with white clover. These interactions remained consistent among harvests but were not always present as significant. There were additional interactions of white clover-red clover in December and plantain-red clover in May.

Table 4.4: Coefficients used in Model 5 from a mixture analysis of acid detergent fibre (ADF) (g/kg DM) per harvest which are significant ($p<0.05$) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B4 shows all coefficients from analysis.

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	223.0	254.2	255.9	276.2	246.5	264.4	282.5	233.5	255.9
P	187.6	219.2	236.2	274.5	276.1	267.6	243.7	194.2	250.3
WC	200.5	224.6	231.3	255.4	250.3	268.1	257.0	198.4	248.4
RC	197.3	241.1	246.0	261.0	255.2	256.9	264.0	225.4	255.0
RG*P	-	-	-	-	-	-	-	-	-
RG*WC	-	-	-	-	-	-	-	-	-
RG*RC	-	-49.1	-	88.9	89.9	-	-	-	46.5
P*WC	-	-	-	-	57.6	-	-	-	31.2
P*RC	-	-	-	-	-	-	-	-53.2	-
WC*RC	-	-	-	57.6	-	-	-	-	-
RG*P*WC	-	-	-	-	-	-	-	-	-
RG*P*RC	-	-	-	-	-	-	-	-	-
RG*WC*RC	-	-	-	-	-	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual weighted NDF concentration showed ryegrass had the highest NDF. There were strong interactions between plantain, white clover and red clover indicated by little change in contour when the three species are included in one contour plot (Figure 4.5). This suggested the optimal pasture mix to minimise NDF is likely to be a mixture of plantain, white clover and red clover.

The model fitted to the contour plots for annual weighted NDF is:

$$\text{NDF (g/kg DM)} = 417.1x_1 + 317.1x_2 + 313.3x_3 + 311.0x_4 + 176.0x_1x_3 - 912.3x_1x_2x_3 \quad \text{(Model 6)}$$

The model had a strong fit (R^2 = 82.26%, R^2 adjusted = 80.99%). The quadratic terms RG*P, RG*RC, P*WC, P*RC and WC*RC, the three species terms RG*P*RC, RG*WC*RC and P*WC*RC and the four-species term were insignificant (p>0.05) so were removed from the model.

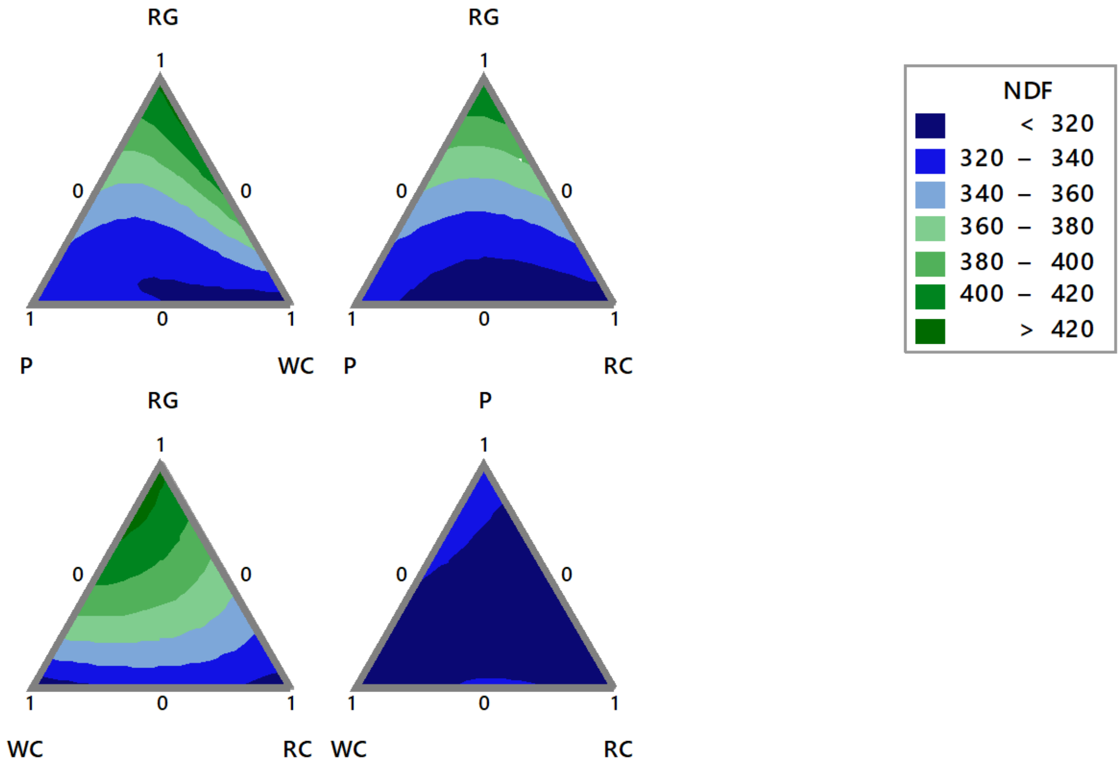

Figure 4.5: Contour plots of annual weighted mean of neutral detergent fibre (NDF) concentration (g/kg DM) from Model 6 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.5) and coefficients (Table 4.5; Table B5) show that ryegrass had the highest concentration of NDF while red clover had the lowest: 417.1 ryegrass > 317.1 plantain > 313.3 white clover > 311.0 red clover g/kg DM. Ryegrass consistently had the highest concentration of NDF at each harvest whilst NDF concentration for the other species varied depending on the harvest date. Additional pairwise interactions can be seen between ryegrass-white clover and ryegrass-plantain-white clover with respect to the annual weighted mean (Table 4.5). These interactions were consistent throughout the harvests, while additional interactions were seen in ryegrass-red clover (August), ryegrass-red clover (September), ryegrass-plantain (October), white clover-red clover (December), ryegrass-plantain-red clover (December) and plantain-red clover (January). Neither white clover nor red clover had an apparent stronger interaction with ryegrass and/or plantain based on the contour plots and coefficients.

Table 4.5: Coefficients used in Model 6 from a mixture analysis for neutral detergent fibre (NDF) (g/kg DM) per harvest which were significant ($p<0.05$) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B5 shows all coefficients from analysis.

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	402.3	436.0	437.7	452.0	408.8	390.6	454.0	406.2	417.1
P	244.4	283.0	282.1	337.0	371.6	348.9	292.3	258.2	317.1
WC	271.6	277.0	293.1	314.0	311.6	347.5	318.5	271.6	313.3
RC	207.0	295.0	292.4	320.0	323.6	328.7	321.1	287.0	311.0
RG*P	-	-	-183.8	-	-	-	-	-	-
RG*WC	-	321.0	-	108.0	140.6	-	224.7	-	176.0
RG*RC	200.5	-224.0	-	-	-	-	-	-	-
P*WC	-	-	-	-	-	-	-	-	-
P*RC	-	-	-	-	-110.1	-	-	-	-
WC*RC	-	-	-	108.0	-	-	-	-	-
RG*P*WC	-	-1730	-	-1000	-927.5	-	-	-	-912.3
RG*P*RC	-	-	-	-926.0	-	-	-	-	-
RG*WC*RC	-	-	-	-	-	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual weighted mean CP concentration showed a decline from the legume (white and red clover) axes to the non-legume (ryegrass and plantain) axes (Figure 4.6). Red clover had a higher contour than white clover, suggesting that the optimal mixture to maximise crude protein concentration is likely to be a red clover monoculture or a mixture of red and white clover.

The model fitted to the contour plots for annual weighted CP is:

$$CP\ (g/kg\ DM) = 146.1x_1 + 141.2x_2 + 208.9x_3 + 234.3x_4 \quad \text{(Model 7)}$$

The model had an adequate fit (R^2 = 74.08%, R^2 adjusted = 73.00%). The quadratic terms, the three species terms and the four-species term were insignificant ($p>0.05$) so were removed from the model.

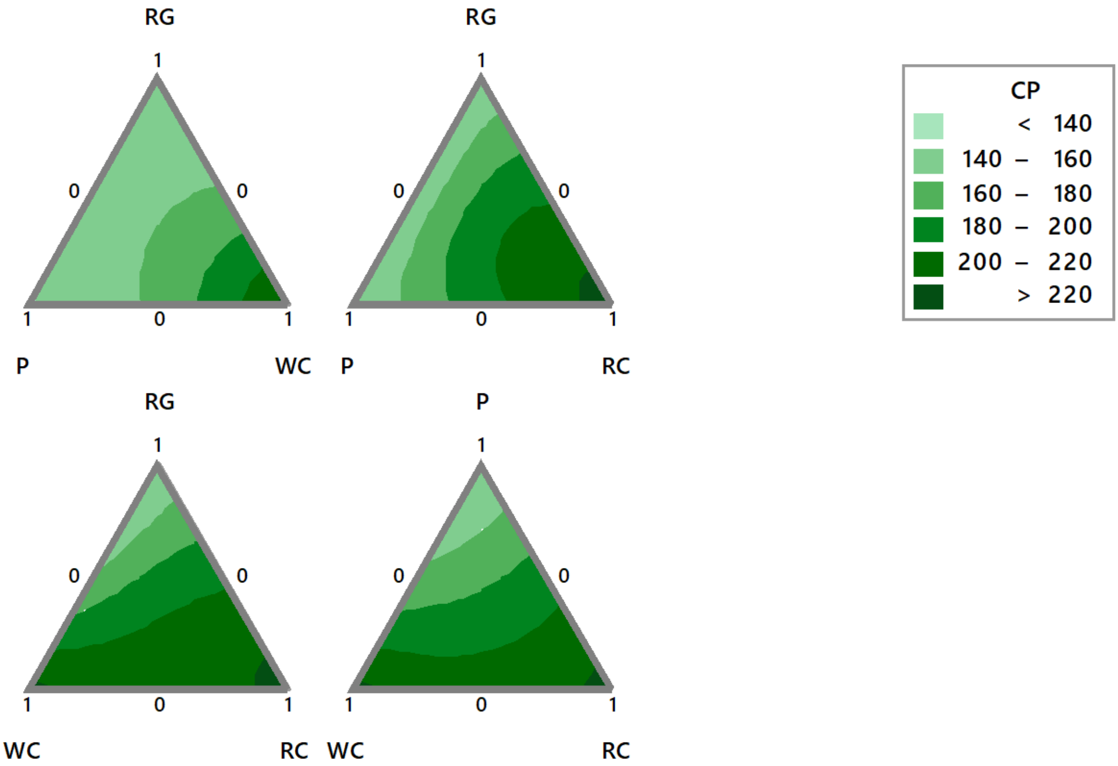

Figure 4.6: Contour plots of annual weighted mean of crude protein (CP) concentration (g/kg DM) from Model 7 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.6) and coefficients (Table 4.6;Table B6) of the monocultures show that red clover crude protein concentration was greatest and plantain was lowest: 234.3 red clover > 208.9 white clover > 146.1 ryegrass > 141.2 plantain g/kg DM. This difference remained consistent throughout the harvests with plantain having the lowest CP concentration except for the August to December period. Comparatively, red clover has the highest CP concentration except for the March and May harvests. There were no additional pairwise interactions in Model 7, but there were interactions with ryegrass and white clover appearing to be interacting the most (four harvests), as well as a plantain white clover interaction (two harvests). Ryegrass and plantain interact more strongly with red clover based on the steepness of the contour

(Figure 4.6) while there are more significant coefficients for both species when combined with white clover.

Table 4.6: Coefficients used in Model 7 from a mixture analysis for crude protein (CP) (g/kg DM) per harvest which are significant ($p<0.05$) from the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Companion Table B6 shows all coefficients from analysis.

	Harvest date								
	2/08	22/09	31/10	9/12	13/01	17/02	30/03	26/05	Annual
RG	140.5	159.5	147.8	129.7	130.8	169.1	152.7	163.1	146.1
P	141.6	168.8	167.4	144.7	101.0	151.7	143.0	139.4	141.2
WC	203.9	246.6	246.9	215.2	195.3	188.3	257.9	245.0	208.9
RC	210.0	253.6	263.2	237.9	217.3	213.2	239.6	227.8	234.3
RG*P	-	-	-	-	-	-	-	-	-
RG*WC	-96.9	-113.2	-	-	-	-	-73.4	-160.4	-
RG*RC	-	-	-	-	-	-	120.4	-	-
P*WC	-	-	-	-102.5	-	-	-112.2	-	-
P*RC	-	-	-	-	119.2	-	-	-	-
WC*RC	-	-	-	-	-	-	-137.5	-	-
RG*P*WC	-	-	-	-	-	-	-	-	-
RG*P*RC	-	-	-	-	-	-	-	-	-
RG*WC*RC	-	-	-	-	-	-	-	-	-
P*WC*RC	-	-	-	-	-	-	-	-	-
RG*P*WC*RC	-	-	-	-	-	-	-	-	-

Annual yield showed upward curvature on the ryegrass-red clover axis (Figure 4.7). This interaction was strongest when plantain was factored into the mixture compared to white clover. The ryegrass-plantain mixture shows the lowest yield, suggesting that the optimal mixture to maximise yield is likely to be a mixture of ryegrass and red clover or ryegrass plantain and red clover.

The model fitted to the contour plot for annual yield is:

Annual yield (kg DM/ha) = $10175x_1 + 7285x_2 + 10651x_3 + 12043x_4 + 21688x_1x_4 + 13859x_2x_3 + 23776x_2x_4$ (Model 8)

The model has adequate fit (R^2 = 62.50%, R^2 adjusted = 59.24%). The quadratic terms RG*P, RG*WC, the three-species term and the four-species term were insignificant (p>0.05) so were removed from the model.

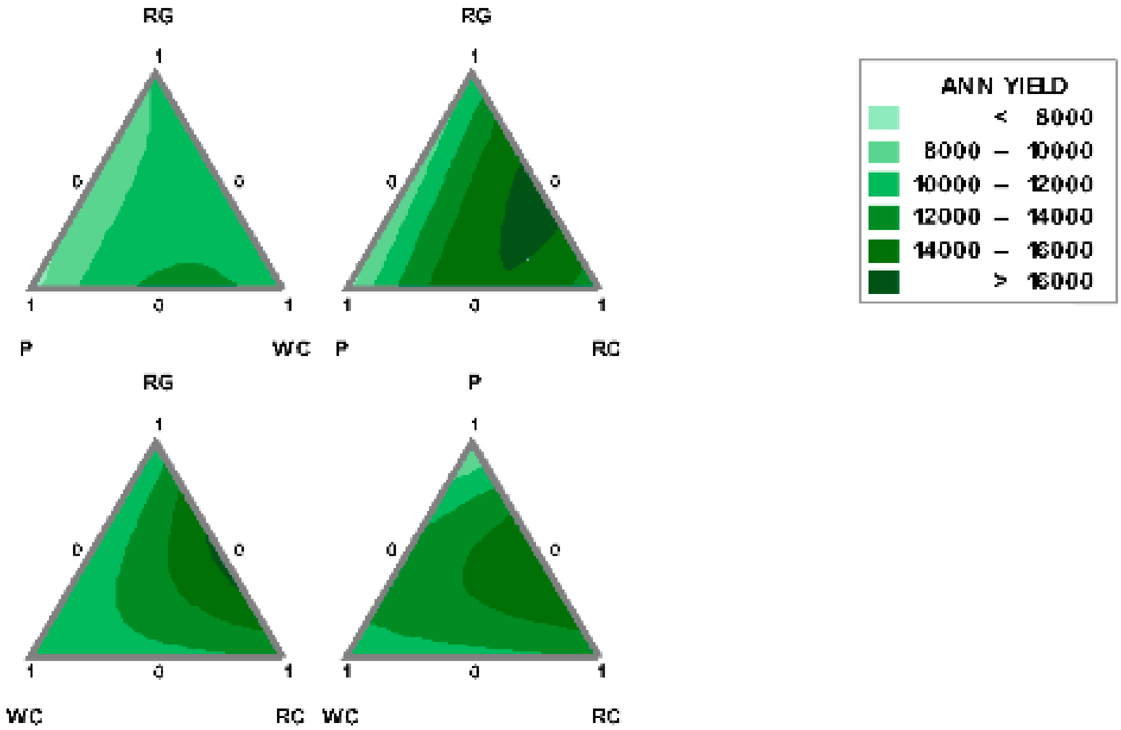

Figure 4.7: Contour plots of annual yield (kg DM/ha) predicted from Model 8 as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plots (Figure 4.7) and coefficients (Model 8) show that red clover has the highest annual yield while plantain has the lowest: 12,043 red clover > 10,651 white clover > 10,175 ryegrass > 7,285 plantain kg DM/ha. The interaction between ryegrass-red clover, plantain-white clover and plantain-red clover (Model 8) can be seen in Figure 4.7 as the increasing curves (darker colours) along the axes of these interactions. Ryegrass and plantain interacted more strongly with red clover rather than white clover based on the steepness of the contours (Figure 4.7) and the significant interactions in Model 8.

4.3 Actual nutritive value compared to modelled

The models produced from analysis can be used to predict nutritive value of any mixture created from the pool of species tested. Figure 4.8 shows the accuracy of the

Model 2 compared to the actual ME yield from NIR analysis. Model 2 fitted the data well, with the majority of the predictions being within the standard error bars of the actual measurement. The predictions of ryegrass and plantain monocultures, the ryegrass-plantain-white clover mixture and the four species even mixture showed high accuracy of prediction. There were potential outliers predicted from Model 2 plantain-red clover and the four species mixture dominated by plantain.

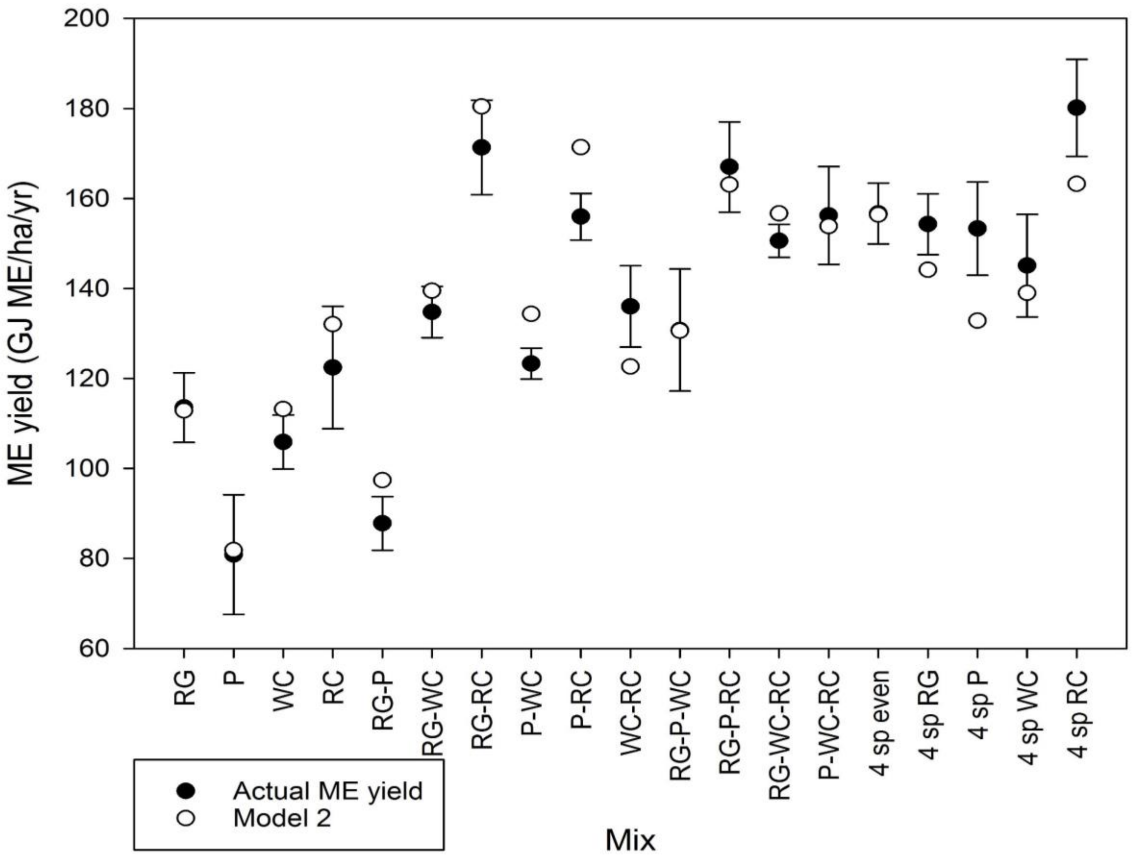

Figure 4.8: Comparison of actual metabolisable energy (ME) yield from analysis and predicted ME yield from Model 2 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

Model 3 can be used to predict the potential CP yield for each of the mixtures based on the sown proportions of each species. Figure 4.9 shows a comparison of the actual CP yield and the prediction based on Model 3. The predictions from Model 3 fit well with the actual levels from analysis. From the predictions, the red clover monoculture, plantain-white clover, ryegrass-plantain-red clover and ryegrass-white clover-red clover fit very similar to the actual values. Otherwise, most of the other predictions of CP yield were within the standard error of the actual values. Potential outliers in Figure 4.9, predicted by Model 3, could be plantain-red clover and the four species mixture dominated by plantain.

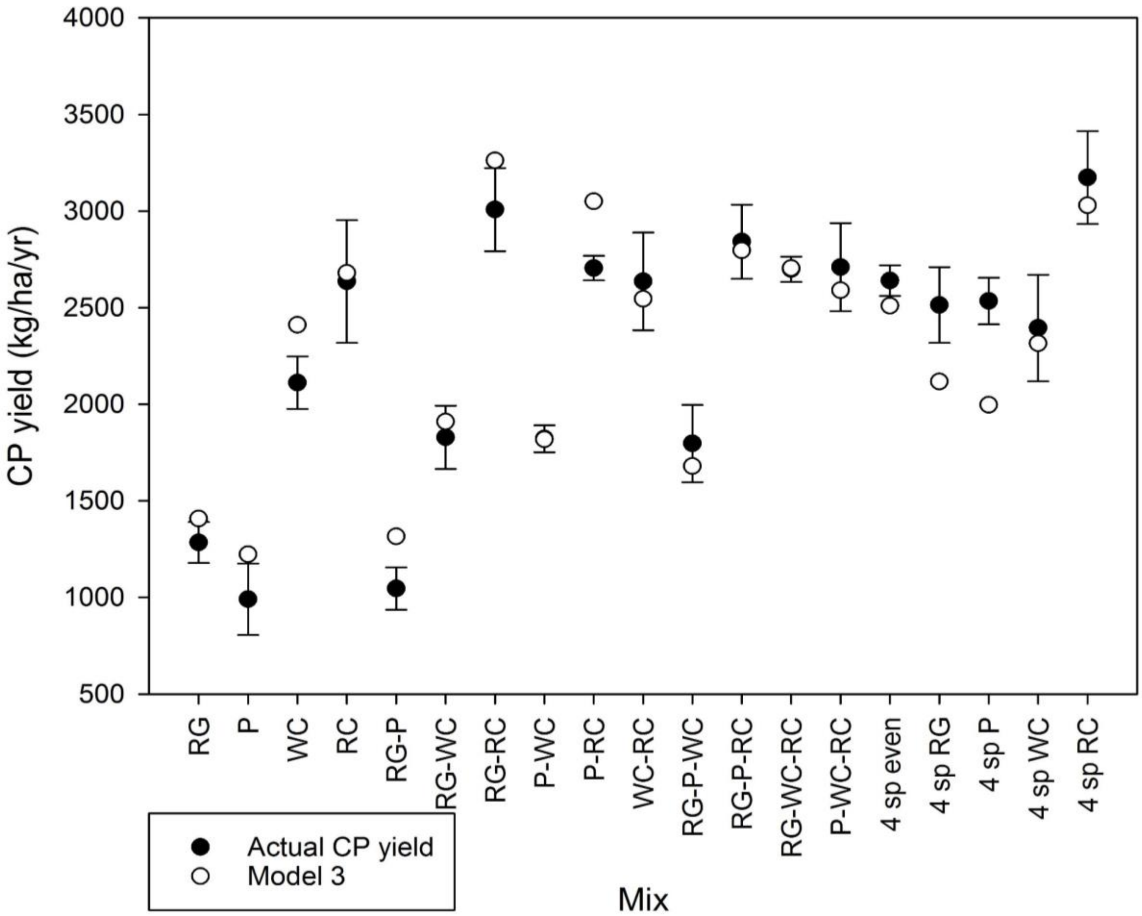

Figure 4.9: Comparison of actual crude protein (CP) yield from analysis and predicted CP yield from Model 3 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

Model 4 can be used to predict the potential ME for each of the mixtures based on the sown proportions of species. Figure 4.10 provides a comparison between the actual values of ME and the predictions from Model 4. The predictions of the red clover monoculture, white clover-red clover, ryegrass-plantain-red clover, ryegrass-white clover-red clover and the four-species red clover dominant mixtures had the most accurate predictions. The prediction for the ryegrass dominant four species mixture appeared to be an outlier due to its predicted ME being much greater than the actual values. Aside from this, most of the other predictions are located within the standard error of the actual values.

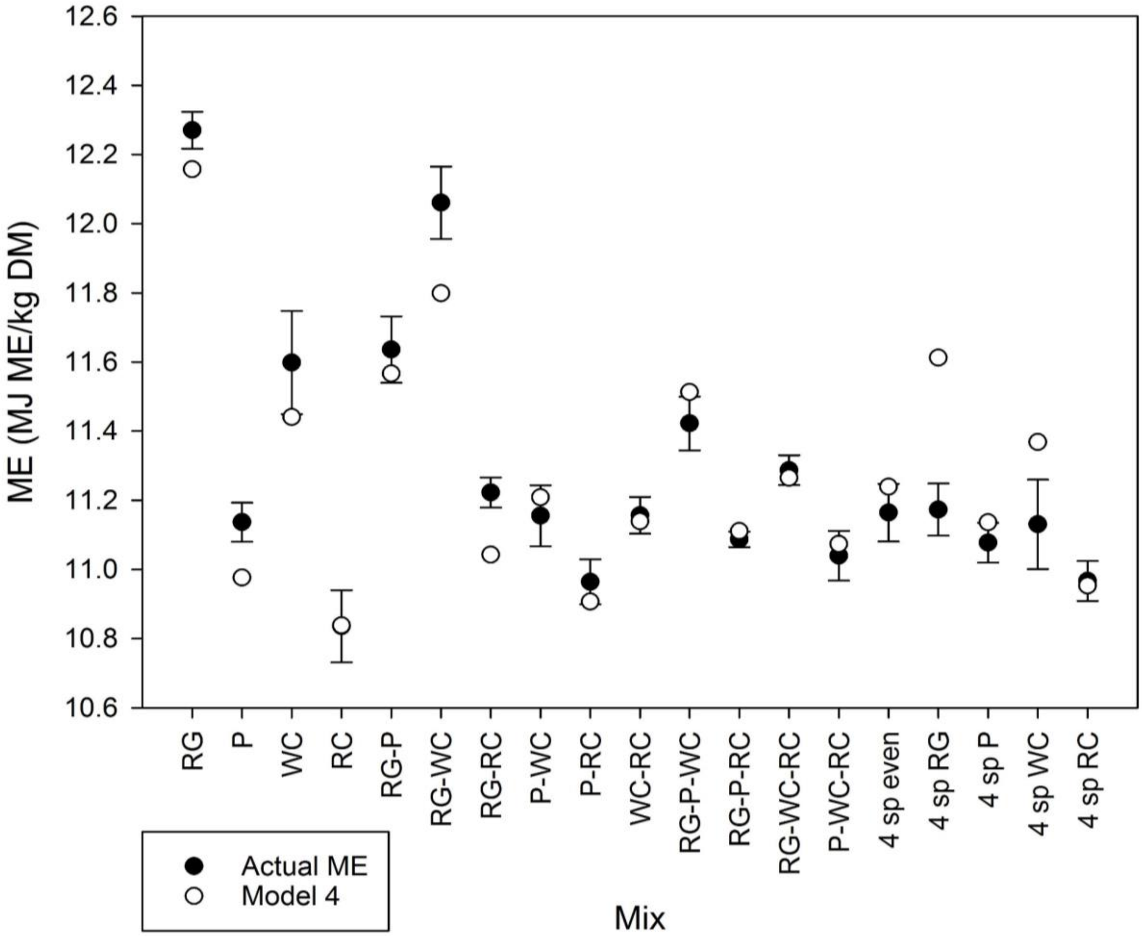

Figure 4.10: Comparison of actual metabolisable energy (ME) concentration from analysis and predicted ME concentration from Model 4 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

Model 5 can be used to predict the potential ADF for each of the pasture mixtures based on the sown proportions of each species. Figure 4.11 shows a comparison of the actual and modelled predictions of ADF. Predictions of ryegrass and plantain monocultures, plantain-white clover, four species even and four species plantain dominant sward have the most accurate predictions from the model. Potential outliers could include the ryegrass-plantain and plantain-white clover-red clover mixtures. The large standard error associated with the ryegrass-plantain mixture could be contributing to this. The large standard error could be caused by the changes in reproductive state of the mixture throughout the year.

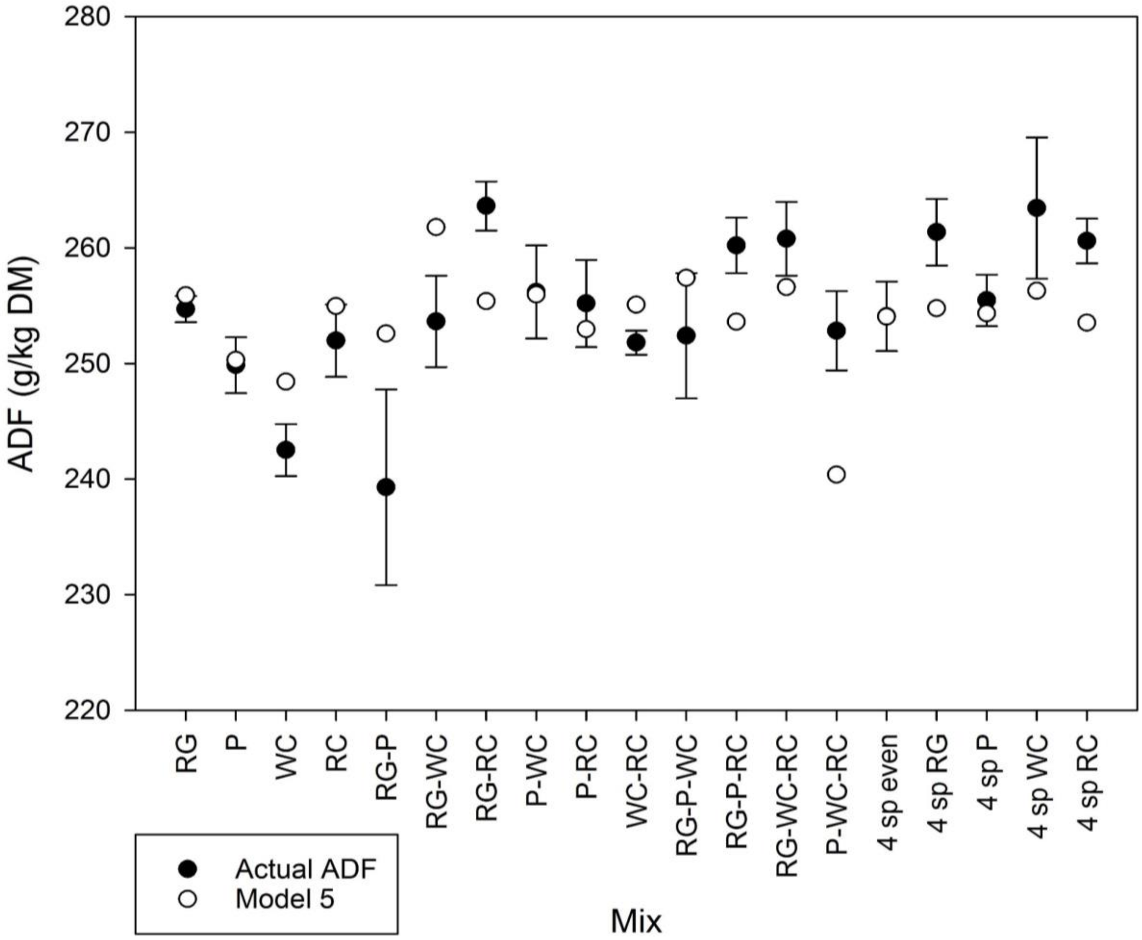

Figure 4.11: Comparison of actual acid detergent fibre (ADF) concentration from analysis and predicted ADF concentration from Model 5 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

Model 6 can be used to predict the potential NDF for each of the pasture mixtures based on the sown proportions of each species. Figure 4.12 shows the comparison of the actual NDF from analysis and a prediction from Model 6. The model shows accurate predictions for red clover monoculture, ryegrass-white clover, plantain-white clover, plantain-red clover, ryegrass-plantain-white clover, four species even and four species red clover dominant mixtures. Most other predictions are within or close to the standard error of the actual measures. There is a potential outlier of the four-species ryegrass dominant mixture.

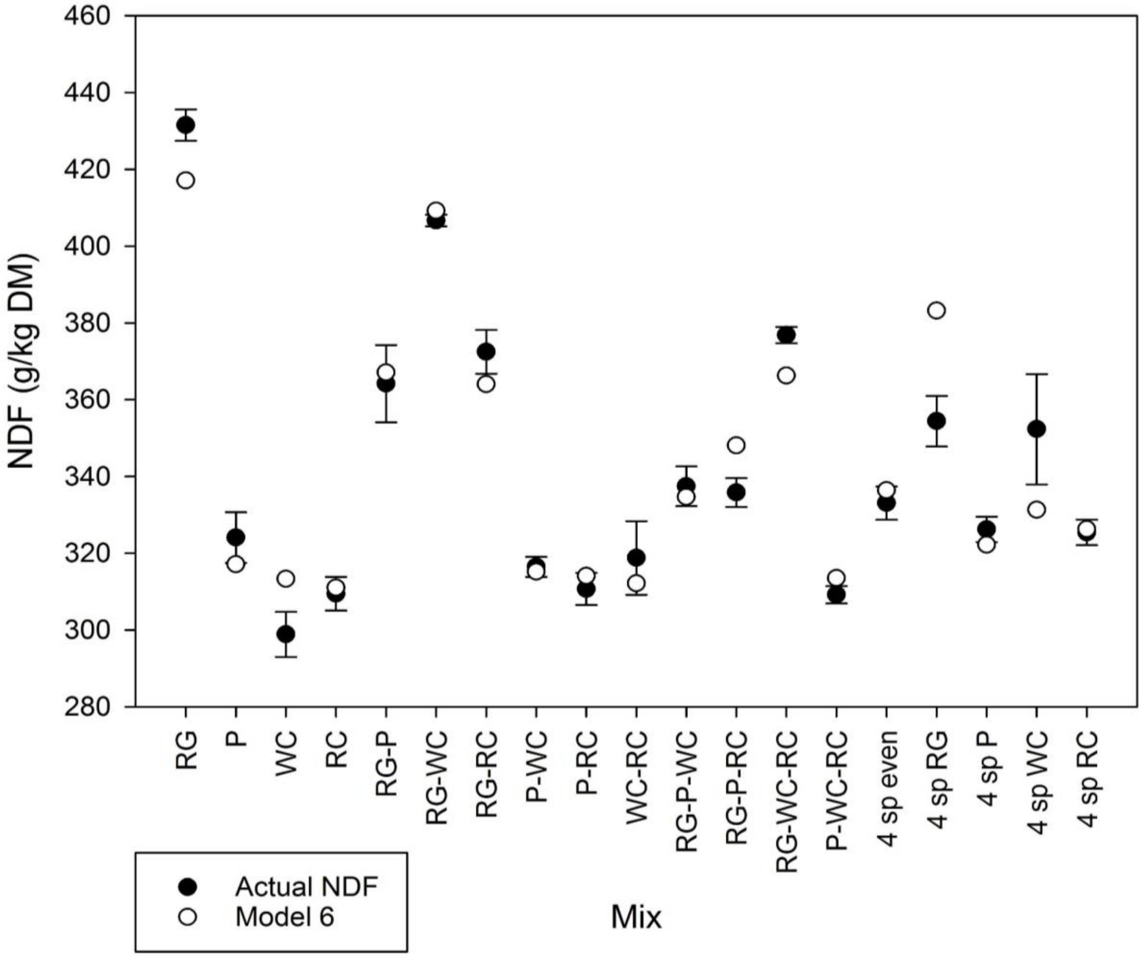

Figure 4.12: Comparison of actual neutral detergent fibre (NDF) concentration from analysis and predicted NDF concentration from Model 6 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

Model 7 can be used to predict the potential CP concentration for each of the pasture mixtures based on the sown proportions of each species. Figure 4.13 shows a comparison of the actual CP concentrations from analysis and predictions from Model 7. The predictions for the red clover monoculture, plantain-red clover were the most accurate predictions. There was some variation between the actual measures and predictions with the white clover monocultures seem to be outliers regarding the accuracy of the prediction.

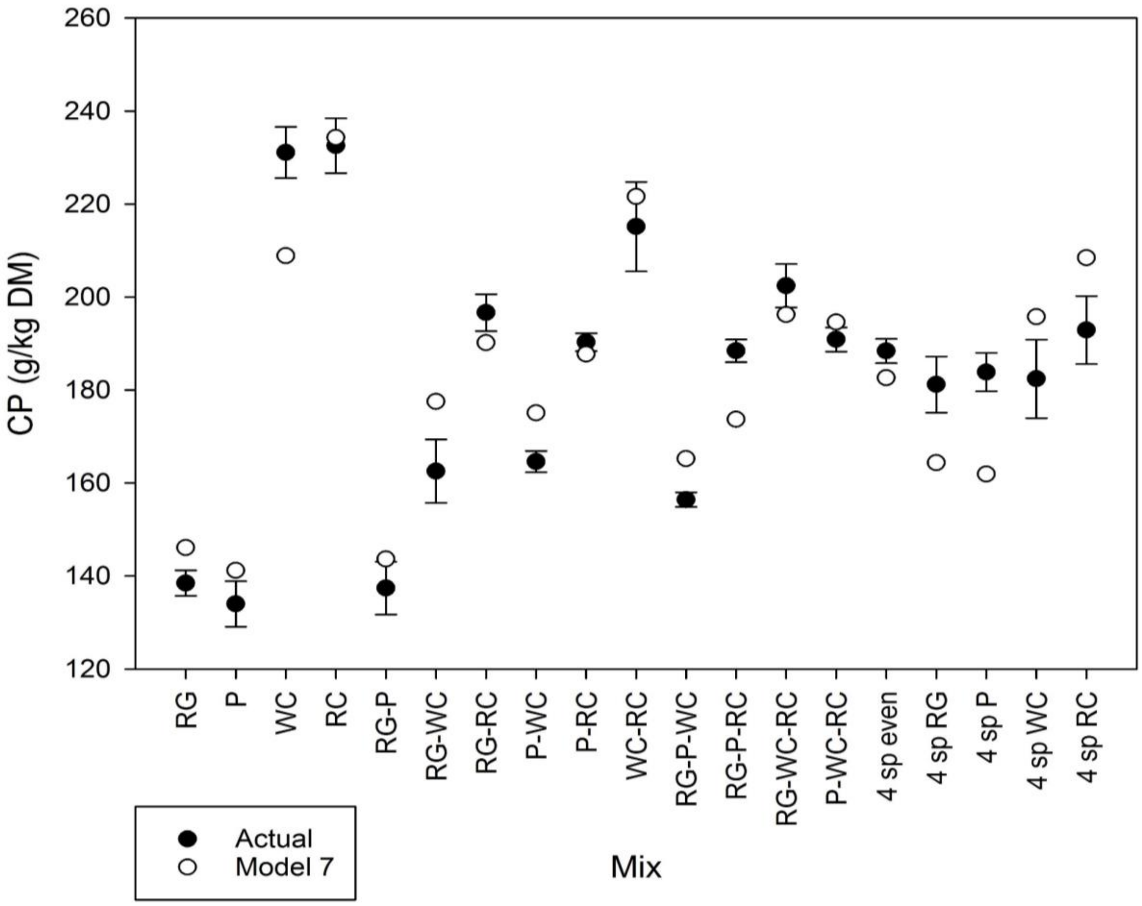

Figure 4.13: Comparison of actual crude protein (CP) concentration from analysis and predicted CP concentration from Model 7 for the mixtures involving the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Error bars denote the standard error surrounding the actual measurements.

4.4 Actual compared to sown proportions of species

The actual proportions of species within the mixtures vary from the sown proportions. The models presented predict the nutritive value of a pasture based on the sown proportions of species in each mixture. Figure 4.14 shows a comparison of the actual and sown proportions of ryegrass, plantain, white clover and red clover. The mixtures showed between a 50% mixture of both plantain and ryegrass to plantain dominant (60-75%). Similarly, ryegrass dominated the ryegrass-white clover mixture making up 70-80% of the sward. In comparison, in the plantain-white clover mixture the plantain comprised approximately 70% of the sward. The ryegrass-plantain-white clover mixture was dominated by plantain at the expense of white clover. Plantain-red clover and ryegrass-red clover mixtures show a dominance towards red clover. Where red clover made up 70% of the sward when mixed with plantain and 50-70% when mixed with ryegrass. A mixture of ryegrass plantain and red clover also showed dominance by red clover reducing the proportion of both ryegrass and plantain. Red clover also dominated in a white clover-red clover mixture making up 80-90% of the sward. While the three-species mixture was more of a 50:50 mixture of ryegrass and red clover, as white clover made up a small proportion of the sward. The three-species mixture showed a similar composition to the 50:50 mixture of ryegrass and red clover. Lastly, the three-species mixture of plantain-white clover-red clover showed very little white clover composition and closer to a mixture of plantain and red clover. This was similar in composition to the 50:50 mixture of plantain and red clover.

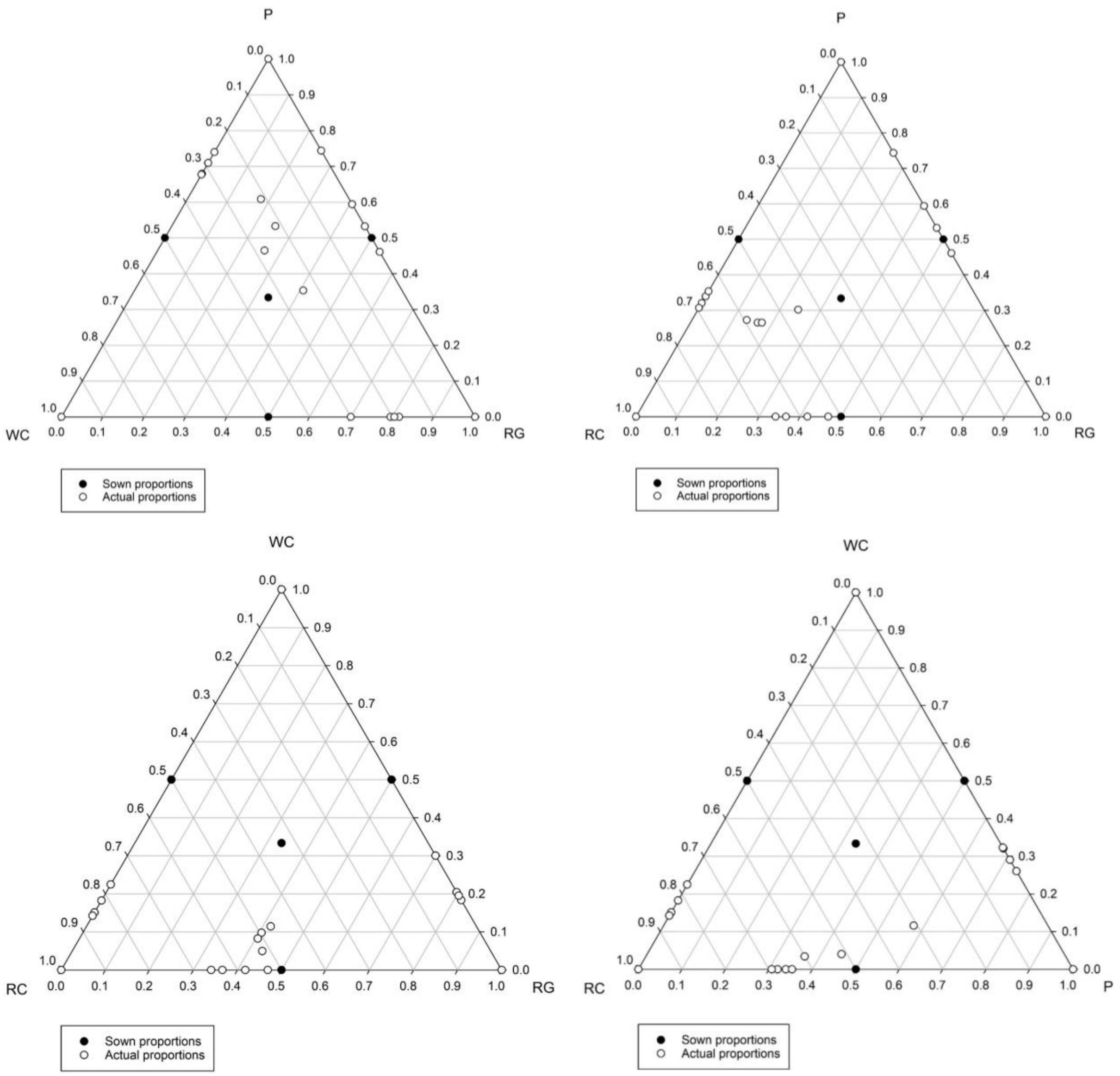

Figure 4.14: Comparison of sown and actual (as a proportion of total yield) proportions of ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) in the sward for each replicate.

The relative abundance of the species changed between the seed mixtures and the annual yield, new models were analysed using the mean annual proportions of species in the annual yield instead of the proportions of species in the seed mixtures to account for how much each species is contributing to the nutritive value of the sward. The adjusted models are:

ME yield (GJ ME/ha/yr) = $121.38x_1 + 78.67x_2 + 110.50x_3 + 128.90x_4 + 169.51x_1x_4 + 204.64x_2x_3 + 226.71x_2x_4$ (Model 9)

$R^2 = 66.23\%$, R^2 adjusted = 63.30%

$$\text{CP yield (kg/ha/yr)} = 1409.5x_1 + 971.3x_2 + 2182.4x_3 + 2642.0x_4 + 3420.6x_1x_4 + 2904.3x_2x_3 + 3553.8x_2x_4 \quad \text{(Model 10)}$$

R^2 = 75.77%, R^2 adjusted = 73.67%

$$\text{ME (MJ ME/kg DM)} = 12.238x_1 + 11.033x_2 + 11.543x_3 + 10.926x_4 - 1.281x_1x_4 \quad \text{(Model 11)}$$

R^2 = 83.97%, R^2 adjusted = 83.06%

$$\text{ADF (g/kg DM)} = 254.78x_1 + 251.70x_2 + 243.05x_3 + 254.20x_4 + 45.16x_1x_4 + 39.77x_2x_3 \quad \text{(Model 12)}$$

R^2 = 33.39%, R^2 adjusted = 28.63%

$$\text{NDF (g/kg DM)} = 431.55x_1 + 317.20x_2 + 301.15x_3 + 310.21x_4 + 57.68x_1x_4 \quad \text{(Model 13)}$$

R^2 = 90.52%, R^2 adjusted = 89.99%

$$\text{CP (g/kg DM)} = 143.6x_1 + 136.4x_2 + 230.8x_3 + 228.4x_4 \quad \text{(Model 14)}$$

R^2 = 87.93%, R^2 adjusted = 87.42%

These models can be used to predict the nutritive value of the swards and compared to the nutritive value from the actual sward and the predictions from models based on the sown proportions of the sward. These models created in a better fit to the actual nutritive value of the sward seen through the higher R^2 for each of the models when compared to the original based on the sown proportions, suggesting that there are fewer outliers producing a more accurate model.

The optimal mixture to maximise yield and nutritive value has a different proportion of ryegrass, white clover and red clover compared to the three species mixture sown in equal proportions. Figure 4.15 shows the difference in sowing proportions of the optimal mixture and the three species mixture in this experiment. The proportions of each of these species in the mixture changes relative to the seasons. The open ends of the joined line represent the first (2/8/16) and last (26/5/17) harvest of the production season. During these cool periods, the ryegrass proportion of the sward is dominant (80%) compared to both clovers (point 1 and 8). The red clover proportion of the sward increases to a maximum of 60% (point 4) while white clover reaches a maximum of 15% (point 3) during the course of the year. Clover proportion in the sward peaks in December and January.

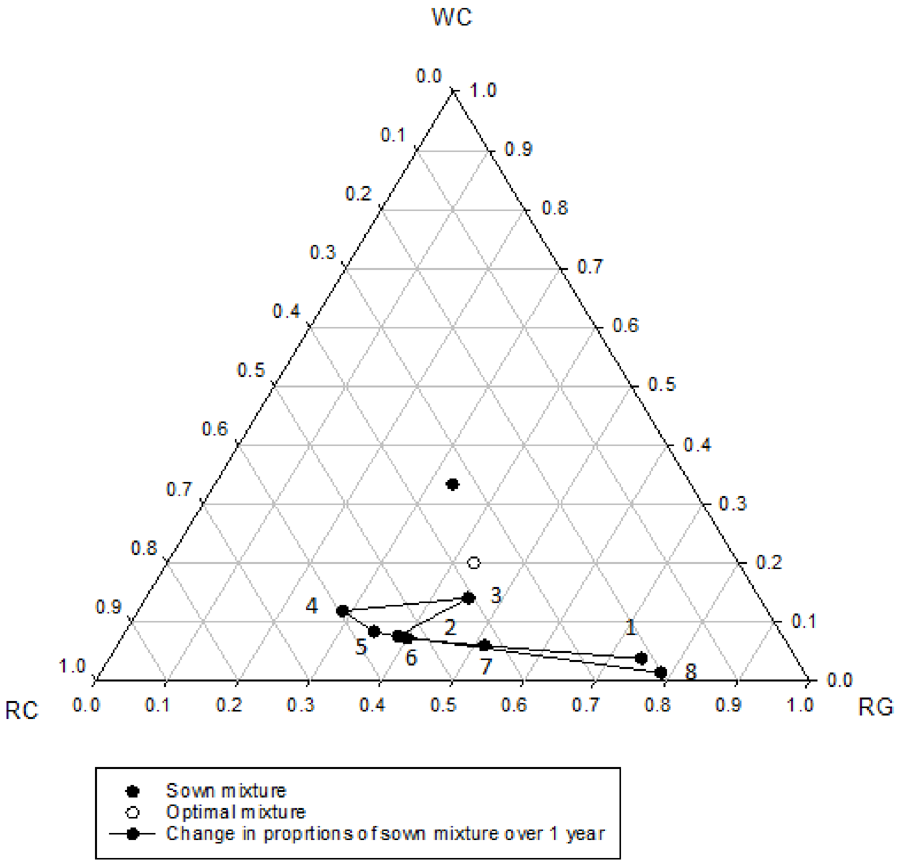

Figure 4.15: Comparison of the proportions of ryegrass (RG), white clover (WC) and red clover (RC) in the optimal mixture, the sown mixture and the change in proportions of the sown mixture from 2/8/16 to 26/5/17.

4.5 Comparison of nutritive value across years

Nutritive value of mixtures changed across time from the previous production season (2015/16) to the full year analysed (2016/17). Based on the monoculture coefficients, ME yield was consistently greater in the 6th January 2016 harvest. Similarly, interactions were also altered in the March harvests. The ME yield of the monocultures was consistently greater in the 31st March 2016 harvest based on the coefficients. Differences in species interactions were apparent through the changes in the significant interactions when comparing the models from the same harvest period. Comparisons show that in 13th January 2017 there were additional interactions between plantain-white clover and white clover-red clover compared to the 6th January 2016 harvest (Table 4.7). Compared to the 31st March 2016 harvest, the 30th March 2017 harvest did not have the ryegrass-white clover interaction, instead there was a ryegrass-red clover interaction. Additionally the plantain-white clover interaction was not significant in the 30th March 2017 harvest (Table 4.7).

Table 4.7: Comparison of metabolisable energy yield coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**28.8**	**21.1**	**18.0**	**8.8**
P	**18.7**	**16.0**	**12.7**	**9.5**
WC	**39.3**	**28.7**	**15.0**	**12.9**
RC	**39.3**	**30.5**	**17.4**	**13.5**
RG*P	15.0	-14.8	-13.8	8.4
RG*WC	**56.2**	**42.1**	**41.8**	26.9
RG*RC	**44.4**	**60.7**	21.2	**39.7**
P*WC	36.4	**48.4**	**39.0**	13.5
P*RC	**73.2**	**56.5**	**48.7**	**44.5**
WC*RC	8.6	**37.0**	-4.9	5.7
RG*P*WC	-127.1	-38.3	-99.1	88.9
RG*P*RC	235.3	60.8	102.8	120.2
RG*WC*RC	107.4	-207.3	31.5	62.9
P*WC*RC	17.0	36.0	46.4	35.7
RG*P*WC*RC	524.5	1364.4	1334.3	-767.9

CP yield did not remain consistent across years. The coefficients from the monocultures in the 13th January 2017 harvest had less CP yields except for ryegrass which was greater than the 6th January 2016 harvest. CP yield of the monocultures was less in the 31st March 2016 harvest than the 13th January 2017 harvest, apart from white clover which is greater than the 30th March 2017 harvest. There was an additional interaction of white clover-red clover in the 13th January 2017 harvest which was absent in the 6th January 2016 harvest (Table 4.8). Additionally, the coefficients of the mixtures for CP yield in the 13th January 2017 harvest are lower for the ryegrass-plantain and ryegrass-plantain-red clover mixtures and plantain, white clover and red clover monocultures but higher for all other mixtures. Alternatively, the 31st March 2016 harvest had an addition interaction in the ryegrass-white clover mixture that was absent in 30th March 2017. The coefficients in Table 4.8 do not show consistent trends when comparing harvests.

Table 4.8: Comparison of crude protein yield coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**182**	**222**	**161**	**108**
P	**180**	**165**	**122**	**120**
WC	**759**	**509**	**274**	**296**
RC	**707**	**591**	**378**	**300**
RG*P	74	-133	-108	139
RG*WC	35	232	**593**	313
RG*RC	**867**	**1073**	**480**	**884**
P*WC	-88	443	262	20
P*RC	**745**	**994**	**470**	**699**
WC*RC	124	**623**	-91	-15
RG*P*WC	-1074	301	-1387	1773
RG*P*RC	2458	1355	192	1729
RG*WC*RC	2923	-1840	476	1258
P*WC*RC	-267	2062	1582	1088
RG*P*WC*RC	4495	12442	9698	-12969

Species interactions of ME concentration were not consistent across years. The ME of the monocultures, based on the coefficients (Table 4.9), were less in the 13th January 2017 harvest compared to the 6th January 2016 harvest. The coefficients of the monocultures were also lower in the 30th March 2017 harvest compared to the 31st March 2016 harvest for all species. There were additional species interactions between ryegrass-white clover, plantain-white clover and white clover-red clover in the 6th January 2016 harvest compared to the 13th January 2017 harvest. A similar trend was seen in the March harvests. Where there were additional interactions between ryegrass-red clover, plantain-red clover and white clover-red clover in the 31st March 2016 harvest which were not present in the 30th March 2017 harvest. The interactions of the mixtures were reasonably consistent across these two harvests based on the similarity of coefficients.

Table 4.9: Comparison of metabolisable energy concentration coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**13.36**	**12.64**	**12.33**	**11.68**
P	**11.31**	**10.69**	**11.52**	**11.31**
WC	**12.19**	**10.68**	**11.96**	**11.50**
RC	**11.14**	**10.75**	**11.18**	**10.76**
RG*P	-0.14	-0.89	0.17	-0.37
RG*WC	**-1.90**	0.44	-1.09	-0.35
RG*RC	**-4.80**	**-2.03**	**-1.72**	-0.95
P*WC	**-2.88**	-1.81	-0.47	-0.01
P*RC	**-2.39**	0.02	-0.06	0.49
WC*RC	**-1.74**	-0.29	**-1.35**	-1.14
RG*P*WC	4.65	-8.56	-4.66	-5.07
RG*P*RC	-1.73	-8.95	-11.12	-2.59
RG*WC*RC	-4.28	-12.06	-0.61	5.15
P*WC*RC	1.73	1.97	-3.49	-6.78
RG*P*WC*RC	59.99	-17.94	21.40	13.53

Species interactions of ADF concentration did not remain consistent between years. The monocultures, based on their coefficients (Table 4.10), remained consistent between years. Comparatively, for the March harvests, the monoculture coefficients were the only significant term from analysis. These remained similar between years but the species interactions were not consistent. There were additional interactions between ryegrass-white clover and plantain-red clover in the 6th January 2016 harvest which were not present in the 13th January 2017 harvest. In the two species mixtures, the coefficients for the 6th January 2016 harvest were greater than the 13th January 2017 harvest, but the three and four species mix were not consistent. Comparatively, the March harvest showed no consistent trends when comparing the mixtures.

Table 4.10: Comparison of acid detergent fibre concentration coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**228.0**	**242.6**	**265.9**	**278.8**
P	**247.0**	**274.9**	**242.4**	**243.4**
WC	**223.0**	**247.5**	**243.3**	**247.0**
RC	**263.0**	**256.9**	**245.8**	**259.6**
RG*P	40.0	13.1	-52.6	-10.0
RG*WC	**155.0**	18.1	28.7	59.1
RG*RC	**153.0**	**67.8**	61.8	0.5
P*WC	**198.0**	**73.9**	17.4	13.3
P*RC	**128.0**	-7.3	43.8	6.2
WC*RC	69.0	2.6	0.4	33.0
RG*P*WC	-494.0	-76.9	104.0	-50.8
RG*P*RC	38.0	173.1	266.6	94.1
RG*WC*RC	215.0	192.1	195.4	69.5
P*WC*RC	-77.0	-281.6	-24.5	152.0
RG*P*WC*RC	-1359.0	1602.5	-845.7	-965.4

Species interactions of NDF concentration did not remain consistent across years. The monoculture coefficients (Table 4.11) showed that the NDF concentrations were greater in the 13th January 2017 harvest. The coefficients for the monocultures remained reasonably consistent between years. There were additional interactions between ryegrass-red clover and plantain-white clover in the 6th January 2016 harvest compared to the 13th January 2017 harvest. Comparatively, the 13th January 2017 had additional interactions between plantain-red clover and ryegrass-plantain-white clover. The other mixtures showed than NDF was lower in the 13th January 2017 harvest for all mixtures expect the four-species mixture. The March harvest showed an additional interaction between ryegrass-plantain in the 31st March 2016 harvest compared to 30th March 2017. While there was no consistent trend for NDF coefficients for the mixtures (Table 4.11).

Table 4.11: Comparison of neutral detergent fibre concentration coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**377.6**	**413.7**	**453.0**	**467.9**
P	**307.7**	**374.0**	**307.0**	**315.2**
WC	**249.2**	**308.2**	**289.0**	**313.6**
RC	**308.6**	**321.7**	**294.0**	**323.2**
RG*P	89.9	-66.1	**-163.0**	-42.2
RG*WC	**405.5**	**126.7**	**180.0**	**234.6**
RG*RC	**158.6**	11.2	100.0	-44.9
P*WC	**237.8**	43.7	2.0	-57.5
P*RC	129.7	**-102.5**	6.0	-54.8
WC*RC	78.0	-6.6	22.0	62.3
RG*P*WC	-781.2	**-963.8**	-303.0	-622.8
RG*P*RC	113.0	-85.2	-304.0	-800.3
RG*WC*RC	695.0	135.1	658.0	373.9
P*WC*RC	-67.8	-221.6	67.0	270.4
RG*P*WC*RC	558.4	2947.8	-4830.0	-464.1

Species interactions for CP concentration did not remain consistent over the years. From the monoculture coefficients, plantain and white clover had greater CP coefficients in the 6th January 2016 harvest while ryegrass and red clover were greater in the 13th January 2017 harvest. The monoculture coefficients showed that the CP concentration was greater for all species in the 30th March 2017 harvest (the same for red clover). There were additional interactions between ryegrass-white clover, ryegrass-red clover and plantain-white clover in the 6th January 2016 harvest which were not present in 13th January 2017. Comparatively, the 13th January 2017 harvest had the interaction of plantain-red clover which was not present in 6th January 2016 (Table 4.12). In the March harvests, there were additional interactions between ryegrass-white clover, ryegrass-red clover and white clover-red clover in the 30th March 2017 harvest (Table 4.12) not present in the 31st March 2016 harvest. The interactions between species in the mixtures shows no consistency between harvests.

Table 4.12: Comparison of crude protein concentration coefficients from analysis between the 2015/16 and 2016/17 growth seasons for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). The coefficients in bold show the significant terms.

	6/01/16	13/01/17	31/03/16	30/03/17
RG	**85.4**	**124.0**	**108.0**	**152.0**
P	**108.1**	**103.0**	**112.0**	**141.0**
WC	**234.0**	**206.0**	**221.0**	**260.0**
RC	**198.5**	**208.0**	**239.0**	**239.0**
RG*P	8.2	7.0	5.0	-4.0
RG*WC	**-151.0**	-71.0	-2.0	**-105.0**
RG*RC	**81.2**	85.0	78.0	**105.0**
P*WC	**-162.0**	-63.0	**-146.0**	**-138.0**
P*RC	-53.7	**99.0**	-78.0	3.0
WC*RC	-43.6	-24.0	-17.0	**-156.0**
RG*P*WC	117.2	288.0	32.0	417.0
RG*P*RC	17.9	266.0	356.0	145.0
RG*WC*RC	196.1	250.0	-287.0	246.0
P*WC*RC	-170.8	326.0	34.0	167.0
RG*P*WC*RC	770.3	-2350.0	-2034.0	-1372.0

4.6 Alternate row treatments

Analysis of the alternate row treatments compared to the species not separated mixtures did not result in a variation in the interactions between species and the nutritive value of the mixture (Table 4.13). Incorporation of the alternative row treatments into the special cubic analysis of the annual ME and CP yields and annual weighted means of ME, ADF, NDF and CP concentrations did not result in differences in the significant species interactions within the models. This meant that the species effects and interactions were consistent across the two sowing methods. However, they did result in a small influence on the coefficients to be used within the models. The alternative row treatments were not further incorporated into the special cubic models due to their lack of influence on the resulting nutritive value components.

Table 4.13: P values from analysis of the alternative row treatments for annual metabolisable energy (ME) and crude protein (CP) yield and annual weighted means for ME, acid detergent fibre (ADF), neutral detergent fibre (NDF) and CP for the mixtures including ryegrass (RG), plantain (P) and white clover (WC).

	p-value					
	ME yield	CP yield	ME	ADF	NDF	CP
Treatment	1.000	1.000	1.000	1.000	1.000	1.000
RG*treatment	1.000	1.000	1.000	1.000	1.000	1.000
P*treatment	1.000	1.000	1.000	1.000	1.000	1.000
WC*treatment	1.000	1.000	1.000	1.000	1.000	1.000
RG*P*treatment	0.982	0.993	0.839	0.882	0.486	0.749
RG*WC*treatment	0.708	0.867	0.260	0.880	0.191	0.201
P*WC*treatment	0.647	0.465	0.964	0.736	0.889	0.340
RG*P*WC*treatment	0.148	0.209	0.500	0.896	0.207	0.975

Proportions of species present in the alternate row treatments (Figure 4.16) showed little variability from the proportions of species sown in the same rows (Figure 4.14). The ryegrass-plantain mixture comprised of between 45% to 70% plantain. The ryegrass-white clover mixture was dominated by ryegrass (65% to 85%). The plantain-white clover mixture was dominated by plantain (60% to 75%). The proportions of species have stabilised at similar proportions, regardless of sowing method (Figure 4.16). The three species mixture showed a similar trend of plantain becoming more dominant in the mixture, at the expense of ryegrass and white clover.

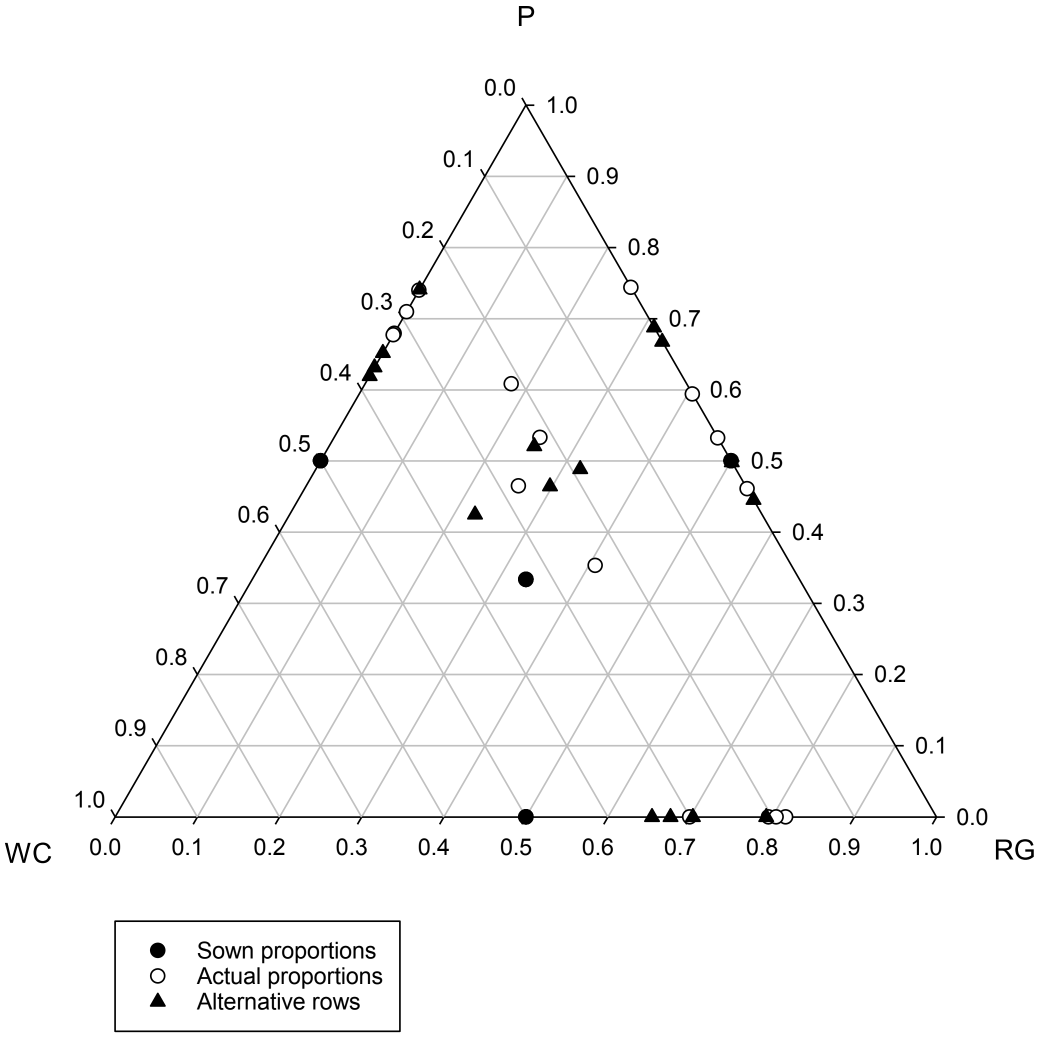

Figure 4.16: Comparison of the sown proportions, species sown in the same row and species sown in alternate rows for ryegrass (RG), plantain (P) and white clover (WC).

5 DISCUSSION

The objective of this experiment was to quantify the nutritive value of pasture mixtures to define an optimal pasture mixture based on the nutritive value characteristics of ME yield, CP yield, ME concentration, ADF concentration, NDF concentration and CP concentration plus the annual dry matter yield. The pasture mixtures could be tested to assess whether nutritive value was stable across seasons and years.

5.1 Optimal mixture

The optimal mixture to meet the defined characteristics of both annual yield and nutritive value (Table 3.3) was 0.43 ryegrass, 0.20 white clover and 0.37 red clover (Plate 3). This was equivalent to 12.90 kg/ha of ryegrass, 1.50 kg/ha of white clover and 6.48 kg/ha of red clover with a total sowing rate of 20.88 kg/ha. The mixture was formulated with respect to the annual yield (DM, ME and CP) and annual weighted mean concentrations of ME, ADF, NDF and CP.

Ryegrass, plantain and red clover mixtures contributed to the high yield of mixtures (annual DM, ME and CP yields) (Figure 4.1; Figure 4.2; Figure 4.7; Plate 4), while ryegrass provided the highest ME (Figure 4.3), plantain and white clover minimised ADF (Figure 4.4), plantain, white clover and red clover minimised NDF (Figure 4.5), while white clover and red clover maximised CP (Figure 4.6). The inclusion of ryegrass in the optimal mixture was likely to be associated with its high yield (annual DM, ME and CP) and high ME. Red clover was likely to be included due to its high yield (annual DM, ME and CP) and high CP. White clover was likely to be included due to its high CP and contribution to reducing ADF and NDF. Plantain has not been included in the optimal pasture mixture. It was included in the mixtures which were suggested to produce the highest annual DM, ME and CP yields, as well as lowering the ADF and NDF. However, when in a monoculture, plantain had the lowest CP concentration (141.2 g/kg DM) and lowest annual yield (7,285 kg DM/ha), which could be a contributing factor to its exclusion from the optimal mixture. Furthermore, Deak *et al*. (2007) showed that CP concentration of a pasture mixture was dependent on the proportion of legume in the mixture, whilst NDF concentration was dependent on the proportion of grass.

The inclusion of clovers in the mixture showed the importance of N supply to the non-legumes. Since the trial was not fertilised with N the legumes were required to supply N. Red clover was 37% of the optimal seed mixture while white clover was 20% of the optimal seed mixture. The high proportion of red clover solidified the greater interaction of ryegrass with red clover rather than white clover. Ryegrass and red clover have less competition for light due to both species having erect structures (Stewart *et al*., 2014). Therefore, ryegrass and red clover have the potential to limit white clover contribution to the sward through shading of the prostrate stems.

5.2 Nutritive value models

The models used to predict nutritive value were based on the annual yields (DM, ME and CP) and the annual weighted mean concentrations (ME, ADF, NDF and CP). Basing models on the annual characteristics of the pasture allowed for the optimal mixture to be the best mixture for annual productivity. Inclusion of three species in the optimal mixture has the potential to maximise both summer and autumn nutritive value. The lack of difference among mixtures in the spring measurements suggested that the variation in differences in nutritive value was not evident until the summer and early autumn harvests.

5.2.1 Changes across seasons

Interactions among species within mixtures were not consistent throughout the harvests. All measures of nutritive value showed that there were no significant interactions of the mixtures during the spring harvests (Table 4.1 to 4.6). During the summer and autumn harvests, species began to show differences in nutritive value, hence the two species mixtures were significant and would be included in a model. The coefficients of the two species mixtures did not give an estimate of nutritive value, they provided a coefficient for multiplication by the proportions of species present in the mixture. Only the monoculture coefficients provided an estimate of the nutritive value of the species.

Mixtures containing three or four-species rarely showed significant interactions with regards to the coefficient terms. The exception of the inclusion of the three-species term of the annual weighted NDF concentration and three significant interactions during the course of the harvest year for ME concentration. The influence of the number of species present in a pasture mixture has been shown by Deak *et al*. (2007) and Sturludóttir *et al*. (2013) to not influence nutritive value. Both studies concluded that the species present and their proportions were the most important factors for determining nutritive value. Comparatively, yield has shown that increases in the number of species present can both increase (White *et al*., 2004) and not alter (Tracy & Sanderson, 2004) yield. However, a similar study based on response optimisation of pasture mixture showed that two species (out of three) produced the highest yielding pasture mixture (Ryan-Salter & Black, 2012). Therefore, location and the species used in the pasture mixture may interact to govern yield and potentially nutritive value response.

5.3 Proportions

5.3.1 Sown compared to actual proportions

The swards used in the trial were in their third year of growth. Figure 4.14 demonstrates the changes in proportions of the sown proportions of species (based on seed count) and the actual proportions of species (based on species yield as a proportion of total yield). Proportions of white clover had been altered the most as a result of competition among species. White clover subsequently made up 10-30% of the sward when it was sown in a two species mix with any other species, 0-10% when sown with red clover in a three species mix and 30% when sown with ryegrass and plantain in a three species mix. The higher proportion when sown with ryegrass and plantain compared to when red clover was also included in the mixture is likely to represent the synergism between white clover and ryegrass and plantain. This is seen through white clover providing N, through N fixation for both of these species. Comparatively, when red clover is also present, red clover also fixes N but has the advantage of growing erect when competing for light.

5.3.2 Changes over years

The models in this study provided an estimate of what the nutritive value could be based on the results of the nutritive value of a 3 year old pasture. However, there was evidence that nutritive value had changed from the second year to the third year (Tables 4.7 to 4.12). This could be a function of the proportions of species changing across time. In most instances, nutritive value was declining when comparing the 2015/16 production year to the 2016/17 production year. ME and CP annual yields showed the largest decline while ADF remained consistent, ME showed a slight decline while CP and NDF showed a slight increase. Therefore, it is difficult to assume that the models for nutritive value which have been constructed will be accurate when used to determine the nutritive value for any year aside from the year they were made for. Sturludóttir *et al*. (2013) showed the effects of species changing across time and the subsequent influence on nutritive value. The NDF of red clover increased from 425 to 460 g/kg DM over a three-year period. Comparatively, white clover NDF fluctuated from 450 to 455 g/kg DM in year two and the reduced to 450 g/kg DM in year three. Red clover CP content showed the opposite trend for the three-year period. It declined from 145 g/kg DM in year one to 120 g/kg DM in year three. White clover CP remained consistent at 140 g/kg DM. The CP and NDF concentrations found by Sturludóttir *et al*. (2013) vary from what was observed in the current study. CP was proportionally lower while NDF is higher in the study by Sturludóttir *et al*. (2013) compared to the current study. This could be attributed to the climatic influence and the difference in cutting regime. The mixtures in this experiment contained two legumes and two non-legumes with the NDF and CP remaining consistent across years regardless of the changes in nutritive value of the individual species. Highlighting that the species contribution is more important than the diversity when investigating nutritive value also reported by Deak *et al*. (2007).

5.3.3 Changes across seasons

Species differ in their growth response to temperature. Figure 4.15 shows the changes of the proportions of species of the ryegrass-white clover-red clover mixture. Perennial ryegrass dominated growth during the cooler temperatures (spring and autumn) while both clovers were more favourable in the warm temperatures (summer). Davidson & Robson (1986) showed than under low N conditions, white clover out yielded grass, particularly under high temperatures as well as perennial ryegrass having a higher yield at low temperatures. The tetraploid ryegrass used in this trial has a higher growth than diploid ryegrass at the same low temperature. This explains why ryegrass can compromise 80% of the sward during the cool temperatures while white clover gets to a maximum of 15% and red clover a maximum of 60% (Figure 4.15). Ryegrass does not become as small proportion of the sward as the clovers due to the synergistic supply of N, reducing the competitive edge shown by the clovers in the study by Davidson & Robson (1986).

The optimal mixture is likely to show a similar pattern of alteration of species composition for the different seasons. The white clover proportion in the optimal mixture was lower than that in the sown mixture. Therefore, the reduction in the white clover proportion of the sward allowed for ryegrass to provide the majority of the nutritive value characteristics and yield, with the two species varying in proportions depending on the temperature.

5.3.4 Future changes

The lifespan of red clover is commonly 2-4 years but can last up to 7 years in favourable conditions (Stewart *et al.*, 2014). Therefore, as red clover begins to die out of the sward, white clover may become more relied upon for N fixation and become more competitive within the sward. However, it is unlikely that white clover will show the same dominance within the sward as red clover due to the competition for light with ryegrass. To compensate for the reduction in red clover, it may be beneficial to sow red clover back into the sward if white clover proportion does not increase.

5.4 Diversity effect

Diversity effects occur as a result of the increase in a measured characteristic above the expected mid-parental mean. Diversity effects in terms of the increase in yield were the most common form of expression. Increases in yield can be seen through the mixing of ryegrass or plantain with both white clover and red clover (Figure 4.7). The interaction with red clover was stronger, hence the higher contour, showing a higher yield. Harris (1968) showed a similar diversity effect by mixing 'Ruanui' perennial ryegrass with varying proportions of clover. However, when mixing species with similar characteristics, there was no diversity effect. Combining ryegrass and plantain in a mixture showed the same influence of no diversity effect.

Nutritive value diversity effects were not as pronounced as the yield diversity effects. The inclusion of coefficients for two species and three species mixtures in Models 2 to 7 suggests that these mixtures may be the result of a diversity effect. In Model 2 this was seen in the ryegrass-white clover, ryegrass-red clover, plantain-white clover and white clover-red clover. When comparing the actual nutritive value and predicted ME yield (Table 4.7) diversity effects were seen between all two species mixtures aside from ryegrass-plantain mixture. In Model 3 the diversity effect can be seen through the CP yield of ryegrass-red clover and plantain-red clover. A similar diversity effect can be seen in Table 4.8 with the same two species mixtures. In Model 4 the diversity effect can be seen through the ryegrass-red clover mixture coefficient being included in the model with the mixture showing a reduction in ME compared to the monocultures. It was difficult to detect this diversity effect in Table 4.9 as the effect is small and had a slight negative impact on ME. The diversity effect in Model 5 can be seen through the inclusion of the coefficients for the mixtures of ryegrass-red clover and plantain-white clover regarding the ADF of the mixture. These effects were evident in the in all mixtures in (Table 4.10). However, the poor fit of the Model may be a cause of this. In Model 6 a diversity effect was seen in the ryegrass-white clover and ryegrass-plantain-white clover mixtures. Table 4.11 shows little diversity effect from these mixtures. In Model 7 no diversity effect can be seen in CP concentration. Table 4.6 did not show a diversity effect either.

The coefficients used within each model showed variation between the models based on the sown proportions of species and those using the actual proportions of species. The additional coefficient terms remained the same for the prediction of ME, ADF and CP but the other models were altered by the influence of the proportions of species. Therefore, the proportions of species present may influence the diversity effect which was similar to the diversity effect of yield seen by Harris (1968) where the increasing proportion of clover resulted in a larger diversity effect.

Diversity effects beyond the two species mixtures were difficult to detect as they may not be caused equally by each species in the mixture. The diversity effects seen through ME and CP yield were easier to detect than the diversity effects from concentration as yield enlarges differences. The lack of diversity effect displayed in the nutritive value concentrations suggests that the importance of the proportions of species present outweighs any potential synergistic effects through mixing species. This indicates that the higher nutritive value of one species cannot significantly increase the nutritive value of another.

Many of the significant terms in Tables 4.1 to 4.6 showed inconsistent inclusion of mixtures in the models for nutritive value. CP concentration (Model 7) does not include any additional terms, aside from the monocultures, ME concentration (Model 4) includes the ryegrass-red clover term, ADF concentration (Model 5) includes the ryegrass-red clover term and the plantain-white clover term and NDF concentration (Model 6) includes the ryegrass-white clover term and the ryegrass-plantain-white clover term. Inclusion of these terms required the nutritive value to be significantly different to the other estimates. The lack of consistent inclusion of two species mixtures in the final model suggests that the monocultures may be sufficient to produce the models used to predict the nutritive value. There has been no previous mixture research conducted to confirm nutritive interactions between species.

5.5 Alternate rows

Sowing species in alternate rows did not influence the nutritive value of the pasture mixtures (Table 4.13). Due to the species not interacting on a nutritive value level, only the proportions of species present can be used to determine nutritive value. There was no significant influence of sowing method on the models used to predict nutritive value. Due to the proportions of species present were similar to the mixtures where species were sown together (Figure 4.16). Hurst *et al*. (2000) showed that sowing species in alternate rows could reduce competition during the establishment phase. However, due to the pasture being 3 years old, all species have had the opportunity to expand and compete for resources, which has the potential for proportions of species to closely resemble that of the mixtures sown in the same rows.

6 GENERAL DISCUSSION

Commercial seed mixtures have a sale point which governs what situation they would be best suited for. The commercial seed mixtures in Table A1 and A2 show the sowing rates of the components of the seed mixtures and sale point for the mixture. Whether it has early spring growth, high palatability or high nutritive value. The models produced in this experiment can be used by seed company agronomists to calculate the potential nutritive value of the pasture mixture they are selling and allow for any potential clients to see whether the mixture that is being sold as having high nutritive value does, in fact, have a higher nutritive value than other mixtures. The models produced from this experiment are limited to the pool of four species used but have the potential to allow for anyone to evaluate their pastures for nutritive value. However, this requires the accurate estimation of the proportions of species within the sward accurately.

Nutritive value of some current commercial seed mixtures included in the pool of four species have been predicted in Table 6.1. The predictions of nutritive value show little variation as the sowing rate and proportion of each of the species has little variation. The legume to non-legume ratio appears to be one of the main governing factors the CP and NDF predictions, as these show the largest variation among mixtures. As the proportions of legumes increases, the CP concentration increases and as the proportion of non-legume increases the NDF concentration increases. The proportion of ryegrass appears to be the main driver of the increase in the concentration in NDF. Farmers should then be able to link the concentrations of each of the nutritive value components to better manage their feed management and promote high quality pastures which are able to increase animal production.

Table 6.1: Predictions of nutritive value (metabolisable energy (ME) yield, crude protein (CP) yield, and concentrations of ME, acid detergent fibre (ADF), neutral detergent fibre (NDF) and CP) of pasture mixtures advertised by seed companies where the species are tetraploid perennial ryegrass (PRG), plantain (P), white clover (WC) and red clover (RC) from Models 9 to 14 based on the sown proportions of species. Proportions of species are based on seeds/m^2 with thousand seed weight from White & Hodgson (1999). Calculations are shown in Table C1.

Mix	ME yield (GJ ME/ha/yr)	CP yield (kg/ha/yr)	ME (MJ ME/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	CP (g/kg DM)
30 kg/ha PRG + 4 kg/ha WC	116.3	1768.3	11.9	249.3	371.0	184.1
25 kg/ha PRG + 5 kg/ha WC	115.2	1846.4	11.8	248.2	357.8	192.9
30 kg/ha PRG + 4 kg/ha WC + 4 kg/ha RC	128.1	2084.4	11.7	252.7	366.7	189.8
24 kg/ha PRG + 1 kg/ha P + 4 kg/ha WC + 4 kg/ha RC	129.4	2140.2	11.6	252.5	358.1	192.6

The optimal mixture identified in this experiment is grown under conditions of no N fertilisation and the application of irrigation is likely to be different to an optimal mixture identified in an irrigated, N fertilised dairy environment. In these conditions, the plantain may become more prolific in the pasture mixture due to the availability of N which will increase its CP concentration. Therefore, plantain may be included in the optimal pasture mixture at the expense of white clover being excluded or the proportions of all species becoming reduced.

New Zealand has numerous different climates, hence it would be beneficial to compare the effect of climate and rainfall on nutritive value. Carrying out the same study investigating the nutritive value of pastures in different locations across the country to evaluate the extent of the impact which the environment has on the nutritive value. Additional species could also be added into the trial design, specifically diploid perennial ryegrass, cocksfoot and chicory which are commonly included in commercial pasture mixtures. The mixture design only allows for the inclusion of four species in a special cubic model. Therefore, at each site, there will need to be several different trials to incorporate all the species. However, the scale of the site needed to carry this out could limit the number of experiments at each site. To combat this, the models produced which predict nutritive value often only present the monocultures and the two species mixtures as significant. Therefore, the trial could be replicated using only the monocultures and two species mixtures. This would create a quadratic design and reduce the required trial space. These new trials should be followed from establishment to 5 plus years to test how the change in composition impacts nutritive value.

Species complementarity during nutritive value trials regarding the time of harvest. Close monitoring of when plants are reaching maturity/optimal grazing time would help to ensure nutritive value is optimal. This would further allow for the pasture mixture formed through the response optimiser to represent the optimal nutritive value for the species present. Therefore, the trial could represent a grazing situation potentially increasing the accuracy of estimating the nutritive value of the trial.

Animals could be incorporated into the trial to assess which aspects of nutritive value have the largest influence on production and preference. The design space may need to be extended to be able to have plots which can support self-contained animals. Ideally, these plots would contain the mixture from the response optimiser scenario where it could be tested whether the animal preference and production mirror the optimal nutritive value of the sward.

6.1 Conclusion

- The optimal pasture mixture for maximising yield, ME yield, CP yield, ME and CP while minimising ADF and NDF was 0.43 ryegrass, 0.20 white clover and 0.37 red clover. This was equivalent to 12.90 kg/ha of ryegrass, 1.50 kg/ha of white clover and 6.48 kg/ha of red clover with a total sowing rate of 20.88 kg/ha.
- Nutritive value was highly dependent on the proportions of species in the pasture mixture. Proportions changed as a result of temperature and succession of species.
- Diversity effects were not obvious when mixing species together to improve nutritive value as nutritive value was not improved beyond the mid-species mean when mixing species.
- Nutritive value predictions and the optimal mixture are likely to be environment dependent. Using these models outside of a Canterbury may not provide accurate predictions of nutritive value but may provide an indication of what nutritive value could be on a pasture mixture including tetraploid perennial ryegrass, plantain, white clover and red clover.

REFERENCES

Al-Mamun, M.; Abe, D.; Kofujita, H.; Tamura, Y.; Sano, H. 2008. Comparison of the bioactive components of the ecotypes and cultivars of plantain (*Plantago lanceolata* L.) herbs. *Animal Science Journal 79*: 83-88.

Black, A.D.; Anderson, S.; Dalgety, S.K. 2017. Identification of optimal pasture mixtures that maximise dry matter yield. *Journal of New Zealand Grasslands 79*: 103-109.

Black, A.D.; Laidlaw, A.S.; Moot, D.J.; O'Kiely, P.O. 2009. Comparative growth and management of white and red clovers. *Irish Journal of Agricultural and Food Research 48*: 149-166.

Black, A.D.; Moot, D.J.; Lucas, R.J. 2006. Spring and autumn establishment of Caucasian and white clovers with different sowing rates of perennial ryegrass. *Grass and Forage Science 61*: 430-441.

Black, A.D.; Murdoch, H.M. 2013. Yield and water use of a ryegrass/white clover sward under different nitrogen and irrigation regimes. *Proceedings of the New Zealand Grassland Association 75*: 157-164.

Brookes, I.M.; Nicol, A.M. 2007. The Protein Requirements of Grazing Livestock. pp. 173-187. *In*: Pasture and Supplements for Grazing Animals. Occasional Publication No. 14 New Zealand Society of Animal Production. Eds. Rattray, P.V.; Brookes, I.M.; Nicol, A.M.

Burke, J.L.; Waghorn, G.C.; Brookes, I.M.; Attwood, G.T.; Kolver, E.S. 2000. Formulating total mixed rations from forages – defining the digestion kinetics of contrasting species. *Proceedings of the New Zealand Society of Animal Production 60*: 9-14.

Burke, J.L.; Waghorn, G.C.; Chaves, A.V. 2002. Improving animal performance using forage-based diets. *Proceedings of the New Zealand Society of Animal Production 62*: 267-272.

Burke, J.L.; Waghorn, G.C.; Brookes, I.M.; Chaves, A.V.; Attwod, G.T. 2006. *In vitro* production of volatile fatty acids from forages. *Proceedings of the New Zealand Society of Animal Production 66*: 50-54.

Connolly, J.; Finn, J.A.; Black, A.D.; Kirwan, L.; Brophy, C.; Lüscher, A. 2009. Effects of multi-species swards on dry matter production and the incidence of unsown species at three Irish sites. *Irish Journal of Agricultural and Food Research 48*: 243-260.

Cornell, J.A. 2002. Experiments with mixtures: Designs, models, and the analysis of mixture data. 3rd Edition. Wiley, Chichester, England. 305 pp.

Cox, J.E. 1978. Soils and agriculture of part Paparua County, New Zealand. New Zealand Soil Bureau Bulletin No. 34. New Zealand Department of Scientific and Industrial Research, Wellington. 128 pp.

Cranston, L.M.; Kenyon, P.R.; Morris, S.T.; Kemp, P.D. 2015. A review of the use of chicory, plantain, red clover and white clover in a sward mix for increased sheep and beef production. *Journal of New Zealand Grasslands 77*: 89-94.

Davidson, I.A.; Robson, M.J. 1986. Effect of temperature and nitrogen on the growth of perennial ryegrass and white clover. 2. A comparison of monocultures and mixtures. *Annals of Botany 57*: 709-719.

Deak, A.; Hall, M.H.; Sanderson, M.A.; Archibald, D.D. 2007. Production and nutritive value of grazed simple and complex forage mixtures. *Agronomy Journal 99*: 814-821.

Deaville, E.R.; Finn, P.C. 2000. Near-infrared (NIR) Spectroscopy: an Alternative Approach for the Estimation of Forage Quality and Voluntary Intake. In: *Forage Evaluation in Ruminant Nutrition*. Eds. Givens, D.I.; Owen, E.; Axford, R.F.E.; Omed, H.M. CAB International, England.

Delaby, L.; Baumont, R.; Aufrere, J.; Peyraud, J.L. 2010. Description and prediction of multi-species pasture nutritive value across the grazing season. *Grassland Science in Europe 15*: 485-487.

Derrick, R.W.; Moseley, G.; Wilman, D. 1993. Intake, by sheep, and digestibility of chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. *Journal of Agricultural Science, Cambridge 120*: 51-61.

Edwards, G.R.; Bryant, R.H.; Smith, N.; Hague, H.; Taylor, S.; Ferris, A.; Farrell, L. 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. *Proceedings of the New Zealand Society of Animal Production 75*: 79-83.

Fulkerson, W.J.; Horadagoda, A.; Neal, J.S.; Barchia, I.; Nandra, K.S. 2008. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Herbs and grain crops. *Livestock Science 114*: 75-83.

Golding, K.P.; Wilson, E.D.; Kemp, P.D.; Pain, S.J.; Kenyon, P.R.; Morris, S.T.; Hutton, P.G. 2011. Mixed herb and legume pasture improves the growth of lambs post-weaning. *Animal Production Science 51*: 717-723.

Harrington, K.C.; Thatcher, A.; Kemp, P.D. 2006. Mineral composition and nutritive value of some common pasture weeds. *New Zealand Plant Protection 59*: 261-265.

Harris, W. 1968. Pasture seeds mixtures, competition and productivity. *Proceedings of the New Zealand Grasslands Association 30*: 143-153.

Harris, W. 2001. Formulation of pasture seed mixtures with reference to competition and succession in pastures. pp. 149-174. *In*: Competition and succession in pastures. Eds. Tow, P.G.; Lazenby, A. CABI, Wallingford, England.

Hayes, R.C.; Dear, B.S.; Li, G.D.; Virgora, J.M.; Conyers, M.K.; Hackey, B.F.; Tidd, J. 2010. Perennial pastures for recharge control in temperate drought-prone environments. Part 1: productivity, persistence and herbage quality of key species. *New Zealand Journal of Agricultural Research 53*: 283-302.

Hoskin, S.O.; Stafford, K.J.; Barry, T.N. 1995. Digestion, rumen fermentation and chewing behaviour of red deer fed fresh chicory and perennial ryegrass. *Journal of Agricultural Science, Cambridge 124*: 289-295.

Hurst, R.G.M.; Black, A.D.; Lucas, R.J.; Moot, D.J. 2000. Sowing strategies for slow-establishing pasture species on a North Otago dairy farm. *Proceedings of the New Zealand Grassland Association 62*: 129-135.

Hutton, P.G.; Kenyon, P.R.; Bedi, M.K.; Kemp, P.D.; Stafford, K.J.; West, D.M.; Morris, S.T. 2011. A herb and legume sward mix increased ewe milk production and ewe and lamb live weight gain to weaning compared to a ryegrass dominant sward. *Animal Feed Science and Technology 164*: 1-7.

Kenyon, P.R.; Kemp, P.D.; Stafford, K.J.; West, D.M.; Morris, S.T. 2010. Can a herb and white clover mix improve the performance of multiple-bearing ewes and their lambs to weaning? *Animal Production Science 50*: 513-521.

Kenyon, P.R.; Morel, P.C.H.; Corner-Thomas, R.A.; Perez, H.L.; Somasiri, S.C.; Kemp, P.D.; Morris, S.T. 2017. Improved per hectare production in a lamb finishing system using mixtures of red and white clover with plantain and chicory compared to ryegrass and white clover. *Small Ruminant Research 151*: 90-97.

Kirwan, L.; Lüscher, A.; Sebastià, M.T.; Finn, J.A.; Collins, R.P.; Porqueddu, C.; Helgadóttir, A.; Baadshaug, O.H.; Brophy, C.; Coran, C.; Dalmannsdóttir, S.; Delgado, I.; Elgersma, A.; Fothergill, M.; Frankow-Lindberg, B.E.; Golinski, P.; Grieu, P.; Gustavsson, A.M.; Höglind, M.; Huguenin-Elie, O.; Iliadis, C.; Jørgensen, M.; Kadziuliene, Z.; Karyotis, T.; Lunnan, T.; Malengier, M.; Maltoni, S.; Meyer, V.; Nyfeler, D.; Nykanen-Kurki, P.; Parente, J.; Smit, H.J.; Thumm, U.; Connolly, J. 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology 95*: 530-539.

Kusmartono; Barry, T.N.; Wilson, P.R.; Kemp, P.D.; Stafford, K.J. 1996. Effects of grazing chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture upon the growth and voluntary intake of red and hybrid deer during lactation and post-weaning growth. *Journal of Agricultural Science, Cambridge 127*: 387-401.

Lee, J.M.; Hemmingson, N.R.; Minnee, E.M.K.; Clark, C.E.F. 2015. Management strategies of chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*): impact on dry matter yield, nutritive value characteristics and plant density. *Crop and Pasture Science 66*: 168-183.

Levy, E.B. 1970. Grasslands of New Zealand. 3rd Edition. A.R. Shearer, Govt Print, Wellington, New Zealand. 374 pp.

Lindsay, C.L.; Kemp, P.D.; Kenyon, P.R.; Morris, S.T. 2007. Summer lamb finishing on forage crops. *Proceedings of the New Zealand Society of Animal production 67*: 121-125.

Litherland, A. J.; Lambert, M. G. 2007. Factors affecting the quality of pastures and supplements produced on farm. pp. 81-96. *In*: Pasture and Supplements for

Grazing Animals. Occasional Publication No. 14 New Zealand Society of Animal Production. Eds. Rattray, P.V.; Brookes, I.M.; Nicol, A.M.

Litherland, A.J.; Woodward, S.J.R.; Stevens, D.R.; McDougal, D.B.; Boom, C.J.; Knight, T.L.; Lambert, M.G. 2002. Seasonal variations in pasture quality on New Zealand sheep and beef farms. *Proceedings of the New Zealand Society of Animal Production 62*: 138-142.

Marley, C.L.; Fraser, M.D.; Fychan, R.; Theobald, V.J.; Jones, R. 2005. Effect of forage legumes and anthelmintic treatment on the performance, nutritional status and nematode parasites of grazing lambs. *Veterinary Parasitology 131*: 267-282.

McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D.; Morgan, C.A. 2002. *Animal nutrition*. 6th ed. Prentice Hall; Edinburgh, UK.

Moorby, J.M.; Fraser, M.D.; Theobald, V.J.; Wood, J.D.; Haresign, W. 2004. The effect of red clover formononetin content on live-weight gain, carcass characteristics and muscle equol content of finishing lambs. *Animal Science 79*: 303-313.

Nicol, A.M.; Brookes, I.M. 2007. The metabolisable energy requirements of grazing livestock. pp. 151- 172. *In*: Pasture and Supplements for Grazing Animals. Occasional Publication No. 14 New Zealand Society of Animal Production. Eds. Rattray, P.V.; Brookes, I.M.; Nicol, A.M.

Nobilly, F.; Bryant, R.H.; McKenzie, B.A.; Edwards, G.R. 2013. Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. *Proceedings of the New Zealand Grassland Association 75:* 165-172.

Norris, K.H. 1989. NIRS instrumentation. pp 12-16. *In*: Near Infrared Reflectance Spectroscopy (NIRS): Analysis of forage quality. Eds. Marten, G.C.; Shenk, J.S.; Barton, F.E. United States Department of Agriculture, Agriculture Handbook No. 643. pp 110.

Nyfeler, D.; Huguenin-Elie, O.; Suter, M.; Fossard, E.; Lüscher, A. 2011. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment 140*: 155-163.

Penning, P.D.; Rook, A.J.; Orr, R.J. 1991. Patterns of ingestive behavior of sheep continuously stocked on monocultures of ryegrass or white clover. *Applied Animal Behaviour Science 31*: 237-250.

Ryan-Salter, T.P.; Black, A.D. 2012. Yield of Italian ryegrass mixed with red clover and balansa clover. *Proceedings of the New Zealand Grassland Association 74*: 201-2008.

Sanderson, M.A.; Labreveux, M.; Hall, M.H.; Elwinger, G.F. 2003. Nutritive value of chicory and English plantain forage. *Crop Science 43*: 1797-1804.

Scales, G.H.; Knight, T.L.; Saville, D.J. 1995. Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. *New Zealand Journal of Agricultural Research 38*: 237-247.

Sano, H.; Tamura, Y; Shiga, A. 2002. Metabolism and glucose kinetics in sheep fed plantain and orchard grass and exposed to cold. *New Zealand Journal of Agricultural Research 45*: 171-177.

Soder, K.J.; Sanderson, M.A.; Stack, J.L.; Muller, L.D. 2006. Intake and performance of lactating cows grazing diverse forage mixtures. *Journal of Dairy Science 89*: 2158-2167.

Somasiri, S.C. 2014. Effect of herb-clover mixes on weaned lamb growth. PhD thesis, Massey University, Palmerston North, New Zealand.

Somasiri S.C.; Kenyon P.R.; Kemp P.D.; Morel P.C.H.; Morris S.T. 2015a. Mixtures of clovers with plantain and chicory improve lamb production performance compared to a ryegrass–white clover sward in the late spring and early summer period. *Grass and Forage Science 71*: 270-280.

Somasiri S.C.; Kenyon P.R.; Kemp P.D.; Morel P.C.H.; Morris S.T. 2015b. Growth performance and carcass characteristics of lambs grazing forage mixes inclusive of plantain (*Plantago lanceolata* L.) and chicory (*Cichorium intybus* L.). *Small Ruminant Research 127*: 20-27.

Somasiri, S.C.; Kenyon, P.R.; Kemp, P.D.; Morel, P.C.H.; Morris, S.T. 2016. Effect of herb-clover mixes of plantain and chicory on yearling lamb production in the early spring period. *Animal Production 56*: 1662-1668.

Stewart, A.; Kerr, G.; Lissaman, W.; Rowarth, J. 2014. Pasture and forage plants for New Zealand, 4th edition. New Zealand Grassland Association, Dunedin, New Zealand. 139 pp.

Sturludóttir, E.; Brophy, C.; Bélanger, G.; Gustavsson, A.-M.; Jørgensen, M.; Lunnan, T.; Helgadóttir, Á. 2013. Benefits of mixing grasses and legumes for herbage yield and nutritive value in Norther Europe and Canada. *Grass and Forage Science 69*: 229-240.

Suckling, F.E.T. 1960. Productivity of pasture species on Hill Country. *New Zealand Journal of Agricultural Research 3*: 579-591.

Suter, M.; Connolly, J.; Finn, J.A.; Loges, R.; Kirwan, L.; Sebastià, M.; Lüscher, A. 2015. Nitrogen yield advantage from grass-legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Global Change Biology*: 1-15.

Thom, E.R.; Bryant, A.M. 1996. Use of Italian ryegrass on seasonal dairy farms in northern New Zealand. 2. Milk production. *New Zealand Journal of Agricultural Research 39*: 237-244.

Thom, E.R.; Fraser, T.J.; Hume, D.E. 2011. Sowing methods for successful pasture establishment – a review. *Pasture Persistence. Grassland Research and Practise Series 15*: 31-38.

Thom, E.R.; Ritchie, W.R. 1993. Banded versus blanket spraying & drilling pattern. pp. 55-58. *In*: Pasture Renovation Manual. 2nd Edition. Eds. Pottinger, R.P.; Lane, P.M.S.; Wilkins, J.R. AgResearch, New Zealand Pastoral Agricultural Research Institute Ltd, Hamilton. 151 pp.

Tracy, B.F.; Sanderson, M.A. 2004. Productivity and stability relationships in mowed pasture communities of varying species composition. *Crop Science 44*: 2180-2186.

Vasiljević, S.; Ćupina, B.; Krstic, D.; Milošević, B. 2011. Seasonal changes of proteins, structural carbohydrates, fats and minerals in herbage dry matter of red clover (*Trifolium pratense* L.). *Biotechnology in Animal Husbandry 27*: 1543-1550.

White, T.A.; Barker, D.J.; Moore, K.J. 2004. Vegetation diversity, growth, quality and decomposition in managed grasslands. *Agriculture, Ecosystems and Environment 101*: 73-84.

White, J.; Hodgson. J. 1999. New Zealand Pasture and Crop Science. Oxford University Press, Australia. 323 pp.

Woodward, S.L.; Waugh, C.D.; Roach, C.G.; Fynn, D.; Phillips, J. 2013. Are diverse species better pastures for dairying? *Proceedings of the New Zealand Grassland Association 75*: 79-84.

APPENDICES

Table A1: Dairy pasture mixes as advertised by the seed companies Agriseeds, Agricom and PGG Wrightson in their 2017 pasture brochures. Species include perennial ryegrass (PRG) and white clover (WC).

Company and seed mix	Sowing rate	Total	Benefit
Agriseeds:			
Trojan diploid PRG Kotare WC Weka WC	18-22 kg/ha 2 kg/ha 2 kg/ha	**22-26 kg/ha**	Top performing, palatable pasture
Arrow diploid PRG Kotare WC Weka WC	18-22 kg/ha 2 kg/ha 2 kg/ha	**22-26 kg/ha**	High yielding especially at calving
Alto diploid PRG Kotare WC Weka WC	18-22kg/ha 2 kg/ha 2 kg/ha	**22-26 kg/ha**	High performance, persistent pasture
Viscount tetraploid PRG Kotare WC Weka WC	30 kg/ha 2 kg/ha 2 kg/ha	**34 kg/ha**	Quality feed and high yield
Viscount tetraploid PRG Trojan diploid PRG Kotare WC Weka WC	15 kg/ha 10 kg/ha 2 kg/ha 2 kg/ha	**29 kg/ha**	High palatability pasture with extra robustness

Table A1 continued.

Agricom:			
ONE diploid PRG Mainstay WC Tribute WC	20 kg/ha 3 kg/ha 2 kg/ha	**25 kg/ha**	Summer, autumn and winter growth, quality and persistence
Halo tetraploid PRG Mainstay WC Tribute WC	25 kg/ha 3 kg/ha 2 kg/ha	**30 kg/ha**	Strong summer growth, excellent quality
PGG Wrightson:			
Rely diploid PRG Legacy WC Quartz WC	20 kg/ha 3 kg/ha 2 kg/ha	**25 kg/ha**	Reliable performance mix. Can tolerate low soil fertility
Expo diploid PRG Legacy WC Quartz WC	20 kg/ha 3 kg/ha 2 kg/ha	**25 kg/ha**	Persistent, high quality mix. High sugar grass
Base tetraploid PRG Legacy WC Quartz WC	25 kg/ha 3 kg/ha 2 kg/ha	**30 kg/ha**	Maximum production and persistent mix. Requires high soil fertility

Table A2: Sheep, beef and deer pasture mixes as advertised by the seed companies Agriseeds, Agricom and PGG Wrightson in their 2017 pasture brochures. Species include perennial ryegrass (PRG), white clover (WC), red clover (RC), plantain (P), cocksfoot (CF) and chicory (C).

Company and seed mix	**Sowing rate**	**Total**	**Benefit**
Agriseeds:			
Trojan or Rohan diploid PRG Weka WC Apex WC Safin CF	16-20 kg/ha 2 kg/ha 2 kg/ha 2-3 kg/ha	**22-27 kg/ha**	Top performing, palatable pasture
Arrow or Tyson diploid PRG Weka WC Apex WC Tuscan RC	16-20 kg/ha 2 kg/ha 2 kg/ha 6 kg/ha	**26-30 kg/ha**	For systems requiring winter and early spring feed
Alto diploid PRG Weka WC Apex WC Safin CF	16-20 kg/ha 2 kg/ha 2 kg/ha 2-3 kg/ha	**22-27 kg/ha**	Persistent pasture with high carrying capacity and stock performance
Viscount tetraploid PRG Weka WC Apex WC Tuscan RC	30 kg/ha 2 kg/ha 2 kg/ha 4 kg/ha	**38 kg/ha**	High feed value, ideal for finishing
Viscount tetraploid PRG Trojan diploid PRG Kotare WC Weka WC	15 kg/ha 10 kg/ha 2 kg/ha 2 kg/ha	**29 kg/ha**	High palatability pasture with extra robustness

Table A2 continued.

Agricom:			
ONE diploid PRG Tribute WC Relish or Sensation RC Tonic P Choice C	18 kg/ha 3 kg/ha 4 kg/ha 1 kg/ha 2 kg/ha	**28 kg/ha**	Increased summer, autumn and winter growth, quality and persistence
Halo tetraploid PRG Tribute WC Relish or Sensation RC Tonic P Choice C	24 kg/ha 4 kg/ha 4 kg/ha 1 kg/ha 2 kg/ha	**35 kg/ha**	Persistent with the ability to finish lambs over summer/autumn
PGG Wrightson:			
Rely diploid PRG Tekapo CF Quartz WC Hilltop WC	18 kg/ha 2 kg/ha 3 kg/ha 2 kg/ha	**25 kg/ha**	Reliable performance. Can tolerate low soil fertility
Expo diploid PRG Quartz WC Hilltop WC Puna C	20 kg/ha 3 kg/ha 2 kg/ha 2 kg/ha	**27 kg/ha**	Persistent, high quality mix. High sugar grass
Base tetraploid PRG Hilltop WC Quartz WC Puna C	25 kg/ha 2 kg/ha 3 kg/ha 2 kg/ha	**32 kg/ha**	Maximum production and persistent mix. Requires high soil fertility

Table A3: Summary if chemical composition and nutritive value data (dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) of perennial ryegrass.

DM (g/kg DM)	CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (%)	ME (MJ/kg DM)	Reference
188	155		487	77.3		Burke *et al*. (2000)
188	155	255	487		11.0	Burke *et al*. (2002)
190	160		490			Burke *et al*. (2006)
		301		62.9		Derrick *et al*. (1993)
	243	232	489		11.4	Fulkerson *et al*. (2008)
	232	281				Harrington *et al*. (2006)
209	202[a]	251	467		10.3	Hoskin *et al*. (1995)
	186[a]			74.0		Kusmartono *et al*. (1996)
	159		433	63.3	10.3	Lindsay *et al*. (2007)
187	186	363				Marley *et al*. (2005)
163	187	303	510			Moorby *et al*. (2004)
			508	67.3		Scales (1995)
219	188					Suckling (1960)
192	**187**	**284**	**483**	**69.0**	**10.8**	**Average**

In studies which had more than one measurement, measurements were averaged. [a] Calculated as total N x 6.25.

Table A4: Summary of chemical composition and nutritive value data (dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) of plantain.

DM (g/kg DM)	CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (%)	ME (MJ/kg DM)	Reference
	158					Al-Mamun *et al*. (2008)
130	247		283	85.0		Burke *et al*. (2000)
130	250		280		11.7	Burke *et al*. (2006)
		340		48.9		Derrick *et al*. (1993)
	276	282	439		9.5	Fulkerson *et al*. (2008)
	283	256				Harrington *et al*. (2006)
	173	243	373		10.7	Hayes *et al*. (2010)
	141		465			Sanderson *et al*. (2003)
	148	316				Sano *et al*. (2002)
207	164					Suckling (1960)
156	**204**	**287**	**368**	**67.0**	**10.6**	**Average**

In studies which had more than one measurement the values were averaged.

Table A5: Summary of chemical composition and nutritive value data (crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) of white clover.

CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (%)	ME (MJ/kg DM)	Reference
270		260		11.5	Burke *et al*. (2006)
270	228				Harrington *et al*. (2006)
243		269	72.4	11.8	Lindsay *et al*. (2007)
249	223				Marley *et al*. (2005)
230					Suckling (1960)
250	**226**	**165**	**72.4**	**11.5**	**Average**

In studies which had more than one measurement, measurements were averaged.

Table A6: Summary of chemical composition and nutritive value data (dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) of red clover.

DM (g/kg DM)	CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (%)	ME (MJ/kg DM)	Reference
	270		340		11.1	Burke *et al*. (2006)
	218	263				Marley *et al*. (2005)
203	263	276	356	75.5		Moorby *et al*. (2004)
	214					Suckling (1960)
	180	300	383			Vasiljević *et al*. (2011)
203	**219**	**280**	**360**	**75.5**	**11.1**	**Average**

In studies which had more than one measurement, measurements were averaged.

Table B1: Coefficients of ME yield for the 2016/17 harvest season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which were significant (p<0.05).

	Harvest date								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**10.6**	**8.4**	**23.8**	**16.9**	**21.1**	**12.6**	**8.8**	**12.1**	**114.1**
P	**5.2**	**6.8**	**14.9**	**16.9**	**16.0**	**10.0**	**9.5**	**5.0**	**84.0**
WC	**3.9**	**13.3**	**14.5**	**15.7**	**28.7**	**15.6**	**12.9**	**-0.6**	**105.3**
RC	**3.2**	**15.4**	**16.5**	**26.6**	**30.5**	**14.4**	**13.5**	**3.7**	**124.7**
RG*P	-8.8	10.1	-13.8	-8.9	-14.8	-5.9	8.4	-1.4	-36.6
RG*WC	5.5	9.4	0.6	14.0	**42.1**	4.7	26.9	1.0	**100.4**
RG*RC	3.9	1.7	10.8	**55.8**	**60.7**	**49.1**	**39.7**	-3.5	**214.3**
P*WC	2.9	-2.8	11.0	24.6	**48.4**	10.9	13.5	15.5	**120.6**
P*RC	4.6	**27.0**	13.1	**32.6**	**56.5**	**45.9**	**44.5**	-0.3	**218.8**
WC*RC	-0.3	3.2	2.3	9.7	**37.0**	**28.0**	5.7	1.6	88.0
RG*P*WC	54.6	-43.6	23.6	152.5	-38.3	102.8	88.9	-35.5	312.8
RG*P*RC	-2.8	76.4	69.0	135.4	60.8	8.9	120.2	65.7	539.4
RG*WC*RC	4.4	49.1	2.4	-21.9	-207.3	-54.0	62.9	22.9	-191.3
P*WC*RC	106.0	8.3	39.2	65.6	36.0	-40.2	35.7	-18.0	211.3
RG*P*WC*RC	-576.9	558.5	253.2	-14.9	1364.4	458.1	-767.9	-289.6	1113.8

Table B2: Coefficients of CP yield for the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which are significant (p<0.05).

	Harvest date								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**121**	**101**	**273**	**170**	**222**	**198**	**108**	**152**	**1340**
P	**68**	**88**	**218**	**223**	**165**	**137**	**120**	**53**	**1081**
WC	**70**	**262**	**310**	**302**	**509**	**295**	**296**	**-17**	**2078**
RC	**66**	**322**	**373**	**556**	**591**	**310**	**300**	**85**	**2624**
RG*P	-118	94	-110	-88	-133	-111	139	-25	-324
RG*WC	34	-98	35	182	232	-141	313	53	530
RG*RC	89	-128	290	**1097**	**1073**	**727**	**884**	-31	**4203**
P*WC	15	-194	126	238	443	160	20	289	1096
P*RC	27	187	289	**632**	**994**	**575**	**699**	-16	**3589**
WC*RC	-1	55	43	130	**623**	163	-15	27	1038
RG*P*WC	596	-427	220	2236	301	1753	1773	-543	6451
RG*P*RC	123	1532	1660	2335	1355	1407	1729	1187	11716
RG*WC*RC	-175	-217	592	591	-1840	523	1258	308	1408
P*WC*RC	1410	-532	126	816	2062	753	1088	-278	4889
RG*P*WC*RC	-5645	10715	7414	-271	12442	4504	-12969	-3097	19024

Table B3: Coefficients for ME concentration for the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which are significant (p<0.05).

	Harvest								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**12.50**	**12.20**	**12.61**	**1.89**	**12.64**	**11.19**	**11.68**	**12.30**	**12.18**
P	**11.90**	**1.33**	**11.53**	**10.72**	**10.69**	**11.00**	**11.31**	**12.50**	**11.16**
WC	**11.20**	**12.12**	**12.18**	**11.62**	**10.68**	**10.68**	**11.50**	**12.00**	**11.57**
RC	**10.80**	**11.27**	**11.34**	**10.91**	**10.75**	**10.56**	**10.76**	**10.80**	**10.87**
RG*P	0.50	-0.57	-0.30	-1.03	-0.89	-0.96	-0.37	1.30	-0.29
RG*WC	**4.80**	-0.09	-0.51	**-1.83**	0.44	2.74	-0.35	2.40	0.47
RG*RC	2.00	-0.97	-0.51	**-2.85**	**-2.03**	0.03	-0.95	2.50	-1.34
P*WC	**2.50**	0.35	0.06	**-2.03**	-1.81	-0.20	-0.01	0.30	-0.85
P*RC	**3.00**	0.33	0.12	-1.03	0.02	0.36	0.49	1.10	-0.07
WC*RC	-1.40	0.13	-0.14	**-2.21**	-0.29	2.37	-1.14	-1.00	-0.24
RG*P*WC	-6.10	-2.91	-0.37	4.97	-8.56	-9.01	-5.07	-11.40	-5.79
RG*P*RC	13.20	-4.75	-3.74	2.49	-8.95	2.95	-2.59	-1.60	-4.13
RG*WC*RC	27.30	**-9.01**	-3.25	1.26	-12.06	-14.64	5.15	12.30	-5.24
P*WC*RC	16.00	-3.41	0.19	4.57	1.97	-7.80	-6.78	11.30	-0.30
RG*P*WC*RC	-165.50	-11.41	9.74	-18.47	-17.94	35.97	13.53	-127.90	-9.86

Table B4: Coefficients for ADF concentration for the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which are significant (p<0.05).

	Harvest date								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**217.8**	**250.0**	**255.0**	**274.4**	**242.6**	**263.5**	**278.8**	**234.3**	**254.9**
P	**188.1**	**224.0**	**241.0**	**269.0**	**274.9**	**257.6**	**243.4**	**196.4**	**249.6**
WC	**212.3**	**222.0**	**230.0**	**247.2**	**247.5**	**264.8**	**247.0**	**198.4**	**244.6**
RC	**195.3**	**239.0**	**248.0**	**257.0**	**256.9**	**256.3**	**259.6**	**227.2**	**252.1**
RG*P	13.0	3.0	-33.0	9.9	13.1	48.6	-10.0	-47.9	-5.4
RG*WC	-47.7	47.0	36.0	60.5	18.1	-33.5	59.1	5.8	20.8
RG*RC	40.5	-50.0	5.0	**114.9**	**67.8**	-5.5	0.5	**-54.3**	**41.2**
P*WC	-59.1	-34.0	-7.0	60.2	**73.9**	71.7	13.3	-5.2	**40.5**
P*RC	-27.8	4.0	-7.0	49.4	-7.3	10.3	6.2	**-69.6**	17.0
WC*RC	-26.3	16.0	-25.0	**76.5**	2.6	-4.0	33.0	-21.6	18.8
RG*P*WC	228.8	-53.0	-14.0	-304.7	-76.9	-150.9	-50.8	293.5	-53.6
RG*P*RC	399.4	-1.0	53.0	-249.0	173.1	-54.2	94.1	342.9	57.9
RG*WC*RC	-193.1	186.0	49.0	-187.4	192.1	318.1	69.5	19.7	82.4
P*WC*RC	101.8	-17.0	115.0	-39.7	-281.6	-87.4	152.0	-44.0	-80.1
RG*P*WC*RC	987.6	-1886.0	-1737.0	-429.4	1602.5	518.9	-965.4	-662.9	-4.8

Table B5: Coefficients for NDF concentration for the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which are significant (p<0.05).

	Harvest date								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**387.0**	**439.0**	**425.0**	**455.5**	**413.7**	**374.8**	**467.9**	**399.0**	**424.0**
P	**256.0**	**300.0**	**300.0**	**339.3**	**374.0**	**340.1**	**315.2**	**277.0**	**325.8**
WC	**267.0**	**282.0**	**299.0**	**309.0**	**308.2**	**311.5**	**313.6**	**253.0**	**304.4**
RC	**215.0**	**299.0**	**304.0**	**314.0**	**321.7**	**304.6**	**323.2**	**289.0**	**309.6**
RG*P	-1.0	-49.0	**-138.0**	-86.5	-66.1	86.9	-42.2	-124.0	-56.0
RG*WC	138.0	**292.0**	106.0	**108.7**	**126.7**	**213.2**	**234.6**	106.0	**165.4**
RG*RC	184.0	**-210.0**	31.0	45.2	11.2	-5.7	-44.9	-1.0	6.0
P*WC	-71.0	-85.0	-93.0	48.0	43.7	106.2	-57.5	22.0	21.9
P*RC	-26.0	-47.0	-69.0	17.1	**-102.5**	36.7	-54.8	-73.0	-23.8
WC*RC	-76.0	-2.0	-44.0	**129.9**	-6.6	196.7	62.3	-25.0	60.1
RG*P*WC	130.0	**-1221.0**	-355.0	**-874.6**	**-963.8**	-850.5	-622.8	98.0	**-775.9**
RG*P*RC	-54.0	-316.0	-416.0	**-830.5**	-85.2	71.3	-800.3	128.0	-363.5
RG*WC*RC	717.0	224.0	9.0	-99.2	135.1	-211.7	373.9	1036.0	101.2
P*WC*RC	241.0	96.0	47.0	-148.1	-221.6	-256.5	270.4	-38.0	-131.2
RG*P*WC*RC	-4951.0	-4660.0	-3222.0	-711.9	2947.8	227.4	-464.1	-1218.0	-175.5

Table B6: Coefficients for CP concentration for the 2016/17 production season for the species ryegrass (RG), plantain (P), white clover (WC) and red clover (RC). Terms in bold represent the coefficients which are significant (p<0.05).

	Harvest date								
	2/08/16	22/09/16	31/10/16	9/12/16	13/01/17	17/02/17	30/03/17	26/05/17	Annual
RG	**138.7**	**136.0**	**144.9**	**118.6**	**124.0**	**175.2**	**152.0**	**154.9**	**142.2**
P	**122.5**	**162.4**	**166.4**	**137.4**	**103.0**	**146.5**	**141.0**	**140.6**	**139.4**
WC	**215.7**	**256.3**	**262.2**	**224.7**	**206.0**	**199.6**	**260.0**	**257.1**	**227.4**
RC	**219.1**	**234.4**	**256.5**	**229.1**	**208.0**	**221.8**	**239.0**	**229.6**	**227.5**
RG*P	-8.0	10.7	13.3	**17.1**	7.0	-16.8	-4.0	23.1	7.3
RG*WC	**-90.1**	**-141.1**	-67.9	**-49.0**	-71.0	-105.0	**-105.0**	**-157.6**	**-88.9**
RG*RC	-8.5	**194.0**	6.6	**70.8**	85.0	-55.6	**105.0**	25.5	44.2
P*WC	-72.0	-17.0	-56.3	**-118.2**	-63.0	25.3	**-138.0**	-73.3	**-71.4**
P*RC	-53.8	60.2	44.8	**40.5**	**99.0**	-65.7	3.0	-14.8	28.2
WC*RC	-46.1	-31.3	-8.8	**-65.3**	-24.0	-129.8	**-156.0**	-47.5	**-69.2**
RG*P*WC	-97.1	538.4	60.3	**316.5**	288.0	-77.5	417.0	75.2	214.9
RG*P*RC	182.6	303.8	483.4	**315.2**	266.0	725.0	145.0	280.3	351.3
RG*WC*RC	-192.5	1.9	213.4	**430.7**	250.0	640.1	246.0	-336.2	329.3
P*WC*RC	-165.1	-496.5	-588.4	**-31.9**	326.0	513.2	167.0	-26.6	72.2
RG*P*WC*RC	1245.0	2458.2	1492.7	**-226.5**	-2350.0	121.9	-1372.0	3223.6	-166.4

Table C1: Calculation of nutritive value of commercial pasture mixtures based on Models 9 to 14 including the species ryegrass (RG), plantain, (P), white clover (WC) and red clover (RC).

	Sowing rate (kg/ha)	TSW	Seeds/kg	Seeds/m^2	Proportion
RG	30	3.9	256410	769	0.54
P	0	2.0	500000	0	0
WC	4	0.6	1666667	667	0.46
RC	0	1.9	526316	0	0
RG	25	3.9	256410	641	0.43
P	0	2.0	500000	0	0
WC	5	0.6	1666667	833	0.57
RC	0	1.9	526316	0	0
RG	30	3.9	256410	769	0.47
P	0	2.0	500000	0	0
WC	4	0.6	1666667	667	0.40
RC	4	1.9	526316	211	0.13
RG	24	3.9	256410	615	0.40
P	1	2.0	500000	50	0.03
WC	4	0.6	1666667	667	0.43
RC	4	1.9	526316	211	0.14